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COLLECTIVE PROTECTION FACTORS METHODOLOGY DEVELOPMENT USING HIGH CONCENTRATION POLYDISPERSE INERT AEROSOLS: RESULTS OF FY09 TESTING

Robert Doherty
SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
Gunpowder, MD 21010-0068

Michael Williamson
Jana Kesavan
Daryl Jones
RESEARCH AND TECHNOLOGY DIRECTORATE

Deborah Schepers
NOBLIS CORPORATION
Falls Church, VA 22042-4519

Victor Arca
JACOBS ENGINEERING
Eglin Air Force Base, FL 32542-5000

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Collective protection shelters, designed to remove airborne particles using positive-pressure high-efficiency particulate air (HEPA) filtration, are available in various sizes, shapes, and rigidity. A methodology was developed to establish a test protocol for the measurement of protection factors (PFs) in excess of 5,000,000. Two inert polydisperse aerosols were used under static conditions as surrogates for the smaller submicron virus, toxic industrial chemicals, toxic industrial materials, and the larger micron-sized spore and spore clusters. Polyalphaolefin oil at 0.528 µm mass median aerodynamic diameter (MMAD) and sodium fluorescein dye at 2.4 µm MMAD were disseminated using an industry standard ATI-TDA-4B generator and SU1A Spray Systems nozzles, respectively. PFs of 77,000 and 250,000 were achieved using a single HEPA-protected shelter, and they could be enhanced in excess of 246,000 and 5,000,000 using a second in-line installed HEPA. Although the dual HEPA-protected shelter results were based on a lower limit of detection of the instrumentation, several orders of magnitude of protection enhancement are likely, given the availability of appropriate instrumentation and sensitivity. As the PF is a function of particle size, the use of monodispersed aerosols is a logical, though challenging, extension to shelter particulate penetration.

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EXECUTIVE SUMMARY

The purpose of this study was to develop a test protocol for the measurement of collective protection (COLPRO) shelters having protection factors (PFs) in excess of 5,000,000 against particulate aerosols. This was accomplished using high concentration inert aerosols under static conditions (i.e., in the absence of any wind). Two aerosol challenges were selected: (1) a submicron aerosol formed from nebulized polyalphaolefin (PAO), and (2) a larger particle, sprayed aerosol of a fluorescing dye (sodium fluorescein) with a mass median diameter of 2.4 μm and a logarithmic geometric standard deviation of 1.7 μm. The submicron aerosol challenge represents smaller toxic industrial chemicals (TICs) and toxic industrial materials (TIMs), while the larger aerosol represents single and small spore clusters.

A simulated COLPRO shelter, consisting of a modified S280 shelter, was used with M28 simplified collective protection equipment (SCPE) containing a carbon-based vapor adsorber and a single high-efficiency particulate air (HEPA) filter to supply the clean pressurized air. The lower limits of detection (LLDs) for these tests were measured at 1×10^{-4} mg/m^3 of sampled air for the PAO and 3×10^{-6} mg/m^3 for the fluorescent aerosol. Aerosol challenges penetrating the toxic-free area (TFA) using the SCPE pack with its single HEPA filter were measured to be well above these LLDs. Test results for the PAO submicron nebulized aerosol yielded an average PF value of 77,500 with a standard deviation of 4,300. For the larger particle, sprayed fluorescent aerosol, the measured PF was 250,000, similar to previous test results.

Thus, the SCPE pack containing a single HEPA filter did not present a sufficient challenge for the developed test protocol. To challenge the developed test protocol further, and estimating that the access point for the shelter was through the lowest collection efficiency of 99.97% for 0.3 μm particles for the HEPA filter, a secondary HEPA filter was combined in series downstream of the SCPE pack. Thus, the pressurized air would be filtered by HEPA filter twice before entering the shelter or TFA. A single test using the two HEPA filters in series yielded a PF >250,000 for the nebulized submicron aerosol and a PF of 3 test average >5,000,000. These PF values were calculated using the LLD levels because the actual aerosol challenge levels inside the TFA were below detection. As the aerosol challenges entering the TFA were below the LLDs when using the dual HEPA filters in series, the resulting PFs were conservative values or underestimates of the actual values. These results confirmed that the HEPA filter was a point of entry into the TFA for submicron aerosols during a static aerosol challenge. The PF is dependent on particle size, and a test protocol has been developed that can successfully measure PFs >5,000,000. The PF of our test series is an average value over all particle sizes in the challenge aerosol; however, it is actually shown as a function of particle size.

Even with improved aerosol collection monitoring systems capable of establishing LLDs, further advances in HEPA type filters (e.g., ultra-low penetration air [ULPA] filters) might keep the measured aerosol challenge level inside the TFA below even improved LLDs. This would mean that the use of LLDs would be typical and acceptable for PF testing. With LLDs, a controlled leakage or very low level of challenge aerosol could easily be introduced into the TFA by way of an air aspirator device to verify that detection equipment

inside the TFA functions properly. Such a device was used in the present series of tests to verify that equipment worked properly.

Although the PAO submicron aerosol test represented what would happen with smaller TICs and TIMs, its main purpose was to provide a quick and inexpensive, real-time test to find and locate major leakage into the TFA. Such testing is accepted as standard practice within the filter industry. However, the present PAO test protocol would not be successful in measuring the PF if the ambient aerosol concentration inside the TFA exceeded any challenge leakage. This can occur when personnel inside the TFA are actively stirring up and generating aerosols. By extension, any real-time aerosol particle counter or mass monitor that cannot distinguish between ambient and challenge aerosols, where both are very low in concentration, would not be suitable as a real-time device for PF measurement. However, it may be possible in the presence of very high concentrations of monodisperse aerosols, such as polystyrene latex spheres, to use the particle size or its fluorescence to distinguish it from ambient aerosols. In combination with aerosol diluters and high resolution aerosol size spectrometers, carefully calibrated for particle size and aerosol concentration, this might prove successful. The Aerosol Sciences Branch at the U.S. Army Edgewood Chemical Biological Center is considering such a method for real-time PF measurement, but no funding has yet been identified. Using a fluorescing aerosol as our second test aerosol, we were able to get around the problem of identifying challenge leakage from ambient aerosols even at very low concentrations. It was necessary to use filter dosage samplers rather than real-time aerosol particle counters. The difficulty with dosage filters is that they must be analyzed using a laboratory fluorometer after removing the dye from the filter. This process can be completed by qualified personnel within 2–3 h after collection, but it provides no time frame for any leakage of the challenge aerosol into the TFA. For example, brief but harmful bursts of aerosol agent entering the TFA would be averaged over the test window, which may yield an acceptable overall average dosage level; however, personnel inside the TFA may be exposed to lethal momentary dosages.

PREFACE

The work described in this report was authorized under Project No. CA06PRO411 for the Defense Threat Reduction Agency. The work was started in October 2008 and completed in December 2009.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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COLLECTIVE PROTECTION FACTORS METHODOLOGY DEVELOPMENT USING HIGH CONCENTRATION POLYDISPERSE INERT AEROSOLS: RESULTS OF FY09 TESTING

1. BACKGROUND

The methods described in this report outline specific procedures that were used for static challenge testing of a collective protection (COLPRO) system and its components designed for operation in environments contaminated with Chemical, Biological, and Radiological (CBR) substances. The COLPRO systems used in these environments must be tested to ensure that they provide adequate protection against all the toxic substances to which they may be exposed. Using inert aerosols in the tests reported here, we developed methodologies and procedures to evaluate the protection provided by COLPRO systems operated in inhospitable environments. Challenge testing was performed to evaluate and quantify the protective capability of a COLPRO system in terms of a protection factor (PF). This testing is required to ensure that the COLPRO system does not allow biological agents to enter its toxic-free area (TFA) when it is set up and operated in its designed operational configuration. Inert aerosol tests are included as an expedient method for approximating the biological protection of a COLPRO system without incurring the costs and other encumbrances of full biological challenge tests.

As a consequence of the first use of chemical warfare agents in World War I, the U.S. Military has endeavored to find adequate protection against the use of these agents. COLPRO shelter systems were designed to relieve the Warfighter from the heavy burden of individual protective equipment (IPE). Testing on the first shelter systems was initiated to ensure that the gas/particulate filter unit (GPFU) adequately removed the toxic threat from the air used to pressurize the shelter system. The gas filter is constructed of a carbon-based adsorbent that is able to remove most chemical substances. The particulate filter is used to remove all particles, including biological and radiological particles.

2. INTRODUCTION

Since the introduction of chlorine and mustard agent gases in World War I, there has been progressive development in the methods and equipment used for protecting personnel individually and collectively. The development of individual masks and protective outer garments is called IPE, whereas development of sheltering systems for several personnel is called COLPRO (Mears, 1979). Most modern day shelters consist of a liner system (barrier material) constructed of multilaminate plastic material that is joined together with other liner sections (and adapter sections) at the edges and a motor-blower to supply pressurized filtered air.

The current design of a COLPRO shelter consists of a liner system of a barrier material constructed from multilayered flexible plastic sheets joined together with other such panels to form various modular designs. The impermeable barrier material is fitted over a skeletal framework to provide shape and strength. A GPFU is used to supply sufficient air free

of chemical vapor and particulates to the shelter to produce an overpressure of 0.4–0.6 in. of water. This provides a clean air sweep exiting the shelter through the attached air lock or entry/exit portal and potential leakage sites. The combination of positive air pressure in the COLPRO shelter, along with its impermeable covering, forms the basis for the ability of the COLPRO shelter to keep personnel free from CBR contaminants. The balance of this report addresses the development of a testing protocol to quantify the protection level afforded by a COLPRO shelter against static aerosol challenges.

Methods for testing the effectiveness of liner systems must be conducted to ensure that they do not allow direct infiltration of contaminated air into the shelter. Airlocks or protective entrances (PEs) allow for safe entry of personnel into the TFA of the shelter by purging contaminated air, brought into the airlock (during entry operations), with filtered air from the shelter. Testing of entry/exit operations has already been conducted and reported by Blewett (1985).

These tests were performed to develop a test protocol for the measurement of COLPRO system PFs when challenged with high concentrations of inert aerosols with aerodynamic characteristics resembling biological threats.

The figure of merit for quantifying the protection level of a given COLPRO shelter is the PF, which is simply the ratio of the contaminant concentration outside the TFA (referred to as the challenge concentration) to the contaminant concentration inside the TFA (referred to as the TFA sample concentration).

Nonviable inert aerosol challenges were chosen because they are readily available, easy to use, readily quantifiable, safe, and avoid the costs and other encumbrances of using a biological stimulant such as *Bacillus atrophaeus* aka *Bacillus globigii* (Bg). Two test aerosols were selected as aerosol challenges to an S280 shelter with an M28 simplified collective protection equipment (SCPE) pack clean air supply. Polyalphaolefin (PAO) was selected for its submicron size and has been used for testing and certification of filtration systems (Bergman, 1996; Mil-Std 282). Sodium fluorescein was chosen over other candidate inert aerosols because of its high water solubility and characteristic strong fluorescent emission. Sodium fluorescein has a detection threshold of approximately 3 ng when standard laboratory fluorometric techniques are used. Very high aerosol concentrations of these aerosols were generated and used to challenge the shelter to measure the shelter PF. The ATI 2H photometer (Air Techniques International, Owings Mills, MD) is the primary diagnostic instrumentation for PAO submicron aerosols, while the 47 mm diameter glass fiber filters were used for the larger sprayed fluorescent particle aerosols. Other aerosol instruments were used to establish trends in the particle number and mass distribution. Such equipment included a TSI Aerodynamic Particle Sizer (APS) analyzer model 3321 (TSI, St Paul, MN), a TSI model 9310 AeroTrak, a TSI DustTrak model 8530, and a Thermo Scientific Tapered Element Oscillating Microbalance (TEOM [Rupprecht & Patashnick model 1200, Thermo Scientific, Barrington, IL]).

The particular inert, static aerosols chosen are a nebulized PAO as a submicron aerosol challenge. These aerosols are capable of penetrating high-efficiency particulate air (HEPA) quality filters at about 0.03% for a 0.3 μm particle. They can also penetrate a sprayed

aqueous solution of a strongly fluorescent dye with an aerodynamic mass mean diameter (MMD) of 2.4 μm and a log standard geometric deviation of 1.7. The PAO submicron aerosol is used to scan the overall shelter system and readily detects weaknesses in filter seals, seams, and portals. The sprayed aerosol with an MMD of 2.4 μm is more representative of individual biological spores and spore clusters based on aerodynamic size equivalency.

The design goal of this effort was an adequate protocol testing procedure to test COLPRO shelters against aerosol penetration up to a PF of 5,000,000 for above a 2 μm aerosol challenge and this goal was achieved. Selection of a PF above 5,000,000 was calculated using an assumed challenge concentration of 2.5×10^7 organisms/m^3, an exposure of 60 min, an infectious dose of 10 organisms, and an at-rest breathing rate of 32 L/min.

This report only addresses inert static aerosol challenges and does not cover other aspects of COLPRO shelter testing such as chemical vapor penetration, entry/exit testing, and wind-driven aerosol challenges.

3. FACILITIES AND EQUIPMENT

The U.S. Army Edgewood Chemical Biological Center (ECBC) ambient breeze tunnel (Aberdeen Proving Ground, MD]) was chosen for the biological simulant and inert aerosol challenge tests. The tunnel was partitioned with polyethylene sheathing to create a static air "challenge" chamber with dimensions of 14 × 25 × 14 ft (W × L × H) as shown in Figure 1. A separate instrumentation room for operation of aerosol monitoring equipment was adjacent to the chamber, yet it was isolated from the challenge chamber with an observation window and interfacing ports to the test section. The test chamber could operate under negative pressure using a separate filtration system to prevent the biosimulant/inert aerosol particulates from entering the adjacent, occupied instrumentation room.

A rigid modified S280 "mock" shelter (the TFA) with dimensions of 8 × 12 × 8 ft (W × L × H) was placed in the center of the "challenge" chamber and a minimum of 3 ft clearance between the shelter, walls, and overhead surfaces of the challenge chamber was maintained.

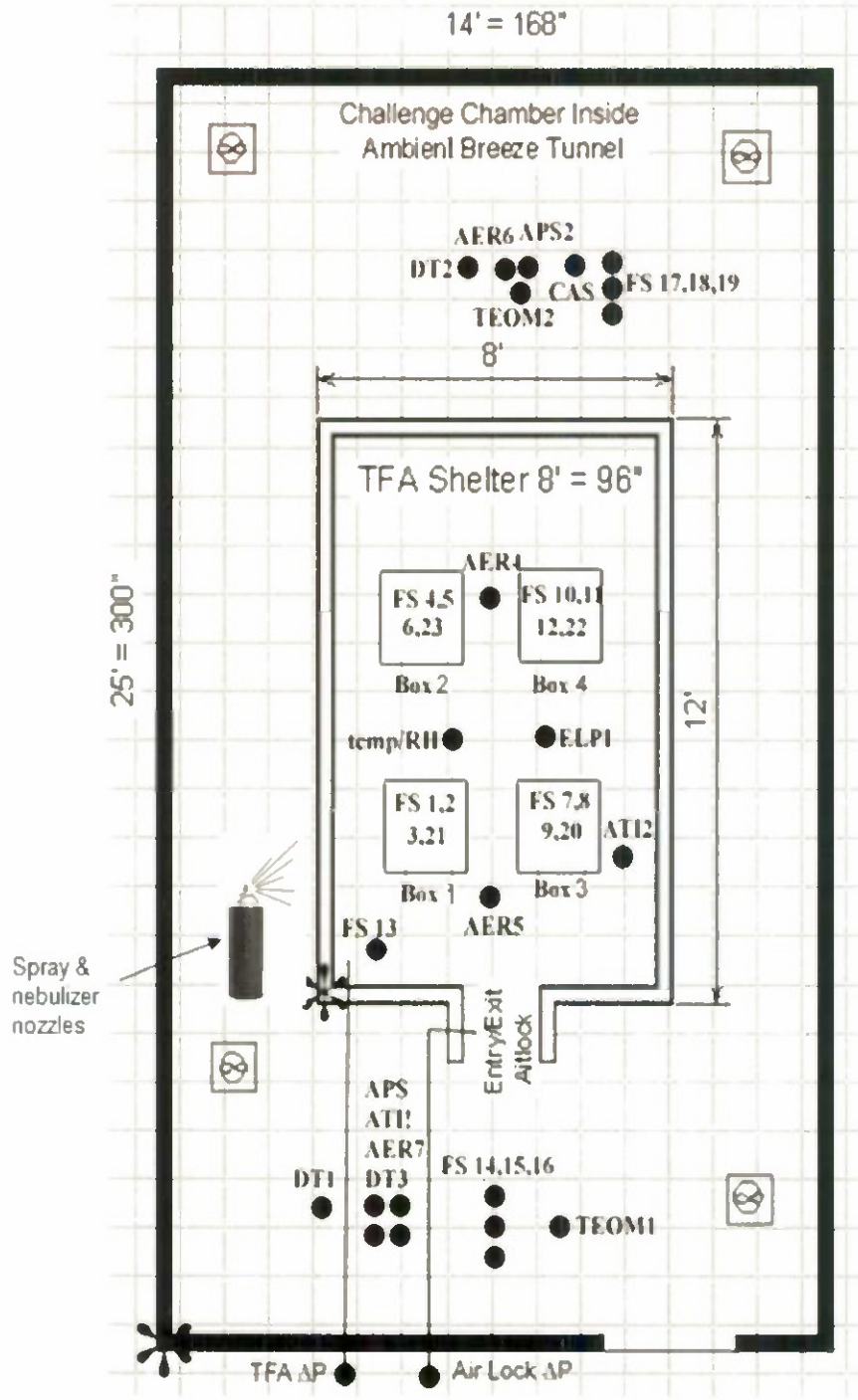

Figure 1. Schematic representation of partitioned segment of ECBC ABT 14 × 25 × 14 ft (W × L × H) illustrating the relative locations of equipment and aerosol monitors used in the static aerosol tests. The TFA dimensions are 8 × 12 × 8 ft (W × L × H).

An external view of the shelter is displayed in Figure 2 with an attached M20 external integrated protective airlock module.

Figure 2. S-280 Shelter and M20 external integrated protective entrance airlock model with an environmental control unit (rear not visible) and an M28 SCPE filter/blower (far left in photo) containing both gas and particulate filters.

Entry to the "mock" COLPRO shelter was through an entry/exit module depicted in Figure 3. The rear view airlock module is visible in the background in Figure 4, further illustrating placement of filter sampling boxes, Electrical Low Pressure Impactor (ELPI, [Dekati, Ltd., Finland]), and meteorological sensors.

Continuous mixing of the aerosol was accomplished using a large fan in each corner of the challenge chamber directed upwards at about 45°. The concentration within the test chamber and TFA was monitored with near real-time and off-line sampling equipment. Dosage filters were used as the truth standard for the sodium fluorescein tests. An APS 3321, AeroTrak 9350, and ELPI were used for real-time monitoring of aerosol particle sizes. An APS 3321, DustTrak 8530, and TEOM were used for monitoring aerosol mass concentrations. The ATI 2H photometer (ATI Inc., Baltimore, MD), successfully used for the PAO submicron aerosol, was not used for the sodium fluorescein as it was not calibrated for that specific analyte. Sample filters were transported to an adjacent laboratory for total mass fluorometric analysis extrapolating from a standard curve.

Figure 3. Entry/exit of TFA.

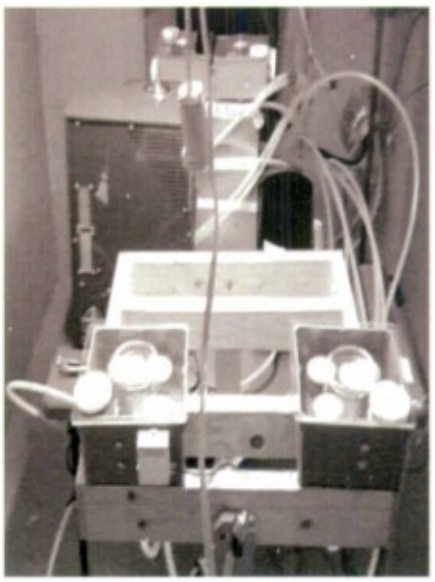

Figure 4. Inside TFA showing equipment platform with rear facing ELPI, closure filter boxes, and temperature/relative humidity meteorological sensors.

The aerosol monitoring equipment is shown in Figure 5 with the relative specifications listed in Table 1. The relative coordinate positioning of the equipment for the challenge chamber and TFA is given in Table 2.

Particle Counters/ Sizers:

APS 3321

AeroTrak 9310

ELPI Impactor

Cascade Impactor

Aerosol Mass Monitors: ATI 2H Photometer, Dusttrak, TEOM

ATI 2H Photometer

TEOM 1200

DustTrak 8530

Filter Dosage Samplers:

47mm Filters

Filter Box

Figure 5. Aerosol monitoring equipment.

Table 1. Acrosol Instrumentation Specifieations for the Equipment Displayed in Figure 5 with Corresponding Coordinate Loeations Provided in Table 2

Description	Manufaeturer	Particle Size Range (μm)	Flow Rate (L/min)	Coneentration
ATI 2H Photometer	ATI	submicron	28.3	0.001–100 mg/m^3
47 mm Filter Holders and Assembly	Hi-Q*	all airborne sizes	10–50	>0.01 mg/m^3
Nonviable 8-Stage Cascade Impaetor	Andersen**	0.4–10 (8 stages)	28.3	>0.01 mg/m^3
APS 3321	TSI	0.523–20 (5 ehannels)	5	1.0×10^3 – 1.0×10^9 partieles/m^3
AeroTrak 9350	TSI	0.3–20 (6 ehannels)	50	$<8.8 \times 10^6$ particles/m^3
ELPI	DEKATI	0.03–10.0 (12 ehambers)	30	>0.01 mg/m^3
DustTrak 8530	TSI	<5	1.4–2.4	280 mg/m^3
TEOM	Rupprecht & Patashnick (Thermo Seientifie)	<10	1	>0.01 mg/m^3
Fluorometer	Sequoia Turner***	single beam with gains of 1, 5, 10, 50, 200, and 1000		

* Hi-Q Environmental Products (San Diego, CA)
** Clean Air Engineering, Inc. (Palatine, IL)
*** Sequoia Turner, Block Scientific (Bohemia, NY)

Table 2. Coordinate Positions for Equipment (Figure 5 and Table 1) Schematically Portrayed in Figure 1. Z-axis is the height in inches above floor level for sampler inlet. Note the separate coordinate origins for the challenge chamber and TFA shelter shown in Figure 1 by enlarged asterisks in the corresponding lower left corners of the chamber and TFA.

CHALLENGE CHAMBER*	x (in.)	y (in.)	z height (in.)
Filter Sample #14	78	30	48
Filter Sample #15	78	30	48
Filter Sample #16	78	30	48
Filter Sample #17	101	264	48
Filter Sample #18	101	264	48
Filter Sample #19	101	264	48
Spray Systems Nozzles	8	168	60
AeroTrak Model 9310 (AER)#6	93	111	41
Aerotrak Model 9310 (AER)#7	68	24	50
ATI Photometer Model 2H #1	73	30	54
DustTrak Model 8530 #1	61	20	46
DustTrak Model 8530 #2	80	25	46
DustTrak Model 8530 #3	61	30	46
APS #1	73	30	54
APS #2	96	264	55
TEOM Model 1200 #1	86	32	68
TEOM Model 1400 #2	97	264	55
Cascade Impactor	108	264	48

TFA**	x (in.)	y (in.)	z height (in.)
Filter Sample #1	50	38	48
Filter Sample #2	50	38	48
Filter Sample #3	50	38	48
Filter Sample #4	44	92	48
Filter Sample #5	44	92	48
Filter Sample #6	44	92	48
Filter Sample #7	64	38	48
Filter Sample #8	64	38	48
Filter Sample #9	64	38	48
Filter Sample #10	61	92	48
Filter Sample #11	61	92	48
Filter Sample #12	61	92	48
Filter sample #13	21	10	64
Filter Sample #20	64	38	48
Filter Sample #21	50	38	48
Filter Sample #22	61	92	48
Filter Sample #23	44	92	48
AeroTrak Model 9310 (AER)#4	52	98	43
AeroTrak Model 9310 (AER)#5	49	30	48
ATI Photometer Model 2H #2	50	38	48
ELPI	63	56	53

* Temp/RH values for x, y, z height in the Challenge chamber were 68, 34, and 45, respectively.
** Temp/RH values for x, y, z height in the TFA were 52, 38, and 48, respectively.

The particular inert, static aerosols chosen were nebulized PAO, disseminated by ATI-TDA-4B (ATI Corp, Baltimore, MD) (Figure 6) as a submicron aerosol challenge. These aerosols are capable of penetrating HEPA quality filters at about 0.03% for a 0.3 μm particle, which is a sprayed aqueous solution of a strongly fluorescent dye disseminated by SS1A (Spraying Systems Corporation, Wheaton, IL) nozzles (Figure 6) with an aerodynamic MMD of 2.4 μm and a log standard geometric deviation of 1.7. The PAO submicron aerosol was used to scan the overall shelter system readily pointing out weaknesses in filter seals, seams, and portals. The sprayed aerosol with an MMD of 2.4 μm is more representative of individual biological spores and spore clusters based on aerodynamic size equivalency.

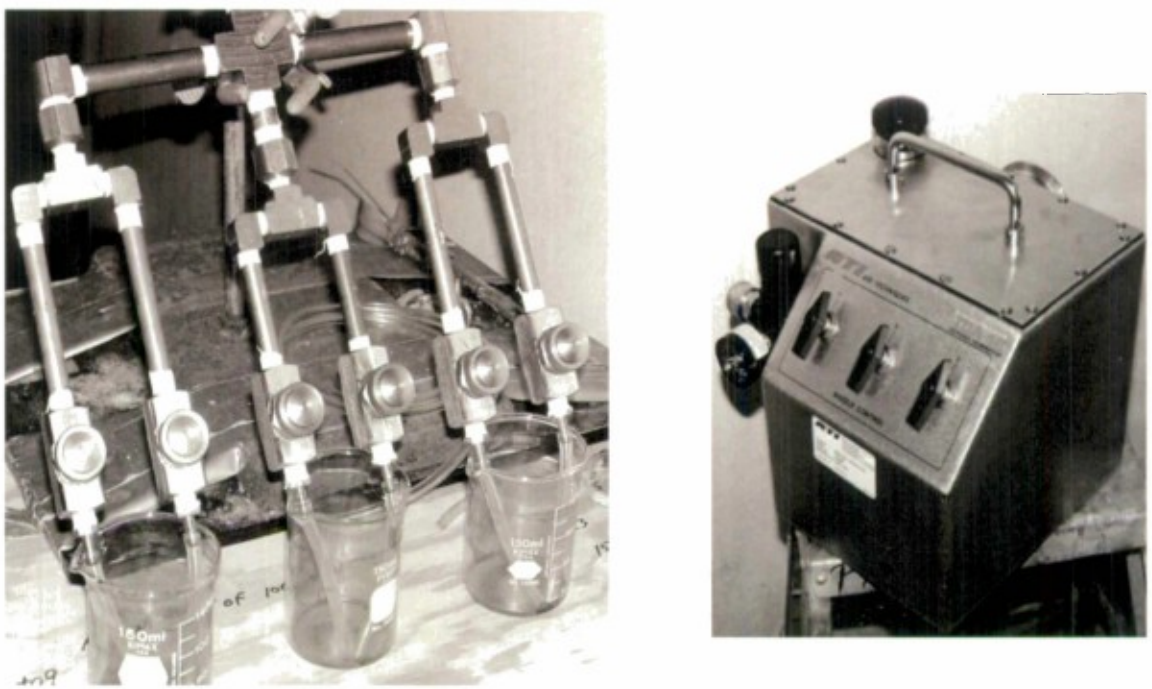

Figure 6. Two commercially available fluid venturi type dissemination SS1A nozzles (left) for sodium fluorescein solutions. The ATI-TDA-4B Laskin nebulizer (right) is designed for complementary use with the ATI 2H aerosol photometer.

The particle size distribution of PAO and sodium fluorescein inert aerosols were monitored using near real-time particle size analyzers. A cascade impactor, outfitted with glass fiber filters coated with a ratio of 2:1 glycerin and water solution (to avoid particle bounce), was used. Total mass concentrations were determined using 47 mm glass fiber filter (Pall Corporation, New Port Richey, FL [type A/E]), analyzed by extraction, and quantitated by standard curve extrapolation using a Sequoia Turner model 450.

3.1 PAO Methodology

The submicron PAO aerosol challenge was disseminated using an ATI Model TDA - 4B nebulizer with six Laskin-style nozzles. PAO liquid has a specific gravity of 0.819 g/mL and, according to its material safety data sheets, it is nonirritating and has no known acute effects. An air pressure of 20 psi for dissemination produces a submicron polydisperse aerosol with number median diameters (NMDs) and MMDs of 0.245 and 0.528 μm, respectively, with geometric standard deviations of 1.65 and 1.55, respectively (ATI operator's manual).

Thus, the PAO serves as a representative small particle threat, which has an inherent penetration of 0.03% through commercial HEPA quality filters. TICS, TIMS, and hosted viruses are likely to be found in this region of the aerosol size spectrum. However, the PAO submicron aerosol was selected to provide a rapid and inexpensive test for identifying major leakage in COLPRO shelter systems and components.

The Model 2H photometer, manufactured and calibrated by ATI, was used to monitor the submicron PAO aerosol. The instrument was calibrated by ATI to yield a full-scale response when challenged with PAO aerosol at 100 mg/m^3. As with all such photometers, calibration is dependent on the particle size, refractive index, and particle shape. Thus, it was advantageous to use the complementary ATI-provided generator-photometer combination in our studies for traceability. Two ATI Model 2H photometers were used to continuously and simultaneously read the challenge chamber and TFA concentrations.

Preliminary testing with the PAO aerosol generator and detector consisted of one person holding the aerosol generator close to a potential leakage site, such as a portal for sampling lines and the intersection of the entry/exit air lock with the shelter, while another person held the sampling line to the photometer directly opposite the generator but inside the TFA. This way, various sample sites were inspected for leakage, but none were found in our S280 shelter. The M28 SCPE was not pretested in this fashion.

To enable accurate PF calculations and to ensure return of background concentrations, especially between trials, 15 min of background data was gathered by the ATI-2H photometers inside the challenge chamber and TFA just prior to the start of a 15 min challenge aerosol generation.

The aerosol mass concentrations (milligrams per cubic meter) were measured in real-time by the ATI-2H photometer. The data was post-processed using Microsoft Excel software and the average mass concentrations inside the challenge chamber and outside the TFA for the background and challenge were calculated. Generally, the PF is the quotient of the average challenge chamber concentration corrected for background divided by the average TFA challenge concentration, which was also corrected for background as shown by eq 1:

$$PF_{PAO} \equiv \frac{\left(\Sigma_{i=1}^{n} c_i t_i / \Sigma_{i=1}^{n} t_i\right)_{\text{Chamber Challenge}} - \left(\Sigma_{i=1}^{m} c_i t_i / \Sigma_{i=1}^{m} t_i\right)_{\text{Chamber Background}}}{\left(\Sigma_{i=1}^{k} c_i t_i / \Sigma_{i=1}^{k} t_i\right)_{\text{TFA Challenge}} - \left(\Sigma_{i=1}^{l} c_i t_i / \Sigma_{i=1}^{l} t_i\right)_{\text{TFA Background}}} \qquad (1)$$

where: n is the number of measurements during chamber challenge
m is the number of measurements during chamber background
k is the number of measurements during the TFA challenge
l is the number of measurements during the TFA background
c_i is the concentration measured at a specific time interval
t_i is the time interval

Note that each average dosage must be normalized by its own time interval. Equation 1 is simplified if all time intervals are equal because then all time frames cancel out.

Although the use of the real-time photometer is emphasized as an expedient method for measuring PF, it is subject to all the same shortcomings of other real-time aerosol particle counters and mass monitors (i.e., it cannot distinguish between ambient and challenge aerosols, has a limited dynamic range for linearity, and its output depends on individual aerosol properties such as particle size, refractive index, and particle shape). Because the photometer and PAO-nebulized aerosol combination has been adopted as an acceptance standard for HEPA quality filter testing, it is used here as a method for rapid determination of major leakage in the COLPRO shelter.

3.2 Sodium Fluorescein

The second challenge aerosol chosen was a sprayed aqueous solution of sodium fluorescein at 15 g/1000 mL of deionized water to represent a bacterial aerosol challenge of single and small spore clusters. This ratio was chosen so that the mass median spray particle from the SS1A nozzle at 60 psig measured about 17 µm, which resulted in a residue particle size of 2–5 µm upon drying. Such an aerosol is well within the respirable size range and has good persistence. The estimated settling velocity of a particle having an aerodynamic diameter of 5 µm is well under 0.1 cm/s. The SS1A spray system pneumatic atomizing nozzle uses a fluid cap (part #1650), along with an air cap (part #64) with a siphon height of 6 in. and a nozzle air pressure of 60 psig. The challenge aerosol was mixed using four room-sized fans in the same manner as the PAO nebulized aerosol. Estimates of aerosol mass concentration were performed using the APS 3321, the DustTrak 8530, and the TEOM 1200. The aerosol generator operator used a third DustTrak to monitor and control the aerosol mass concentration in real-time in an attempt to keep the concentration constant. Aerosol size distributions were monitored by a cascade impactor, APS 3321, AeroTrak model 9350, and ELPI. Because the impactor final filter was mistakenly omitted, the size distribution may have been affected by the loss of as much as 3% of the total mass. The collected aerosol on 47 mm filters is considered as our concentration standard for the following reasons: they are readily analyzed, they have a low fluorescent background, they are readily extractable in recovery solution, they have minimal sampling inlet issues, and they are adaptable to sampling with critical orifices and mass flow controllers. Critical orifices at 10 L/min and mass flow controllers at 50 L/min were selected for the challenge chamber and TFA shelter, respectively.

Measurement of the sodium fluorescein sprayed aerosol was accomplished with filter dosage samplers using a 47 mm diameter glass fiber filter with a collection efficiency minimum of 99.97% at 0.3 μm (Pall Type A/E). The filter dosage samplers were placed in Hi-Q open-faced filter holders. Background aerosol samples inside the TFA that were used in the PF calculation were taken 30 min prior to aerosol challenge generation. As the challenge chamber aerosol concentration was >4 orders of magnitude than the challenge chamber background (background concentration measurements were not required).

Samples acquired during the aerosol challenge period were also 30 min in duration and generally commenced at the onset of aerosol generation. Critical orifice flow measurements for the challenge chamber filters were checked just prior to each test. The average flow rate was 9.2 L/min. Flow rates for the TFA filter samplers were wirelessly monitored and controlled by mass flow controllers.

Minimization of background concentrations on the filters inside the TFA was achieved using covered sampling boxes automating opening and closing and reducing handling. The box lids were remotely opened at the beginning of the test cycle and immediately closed thereafter. Each box lid had a "fallout" filter mounted in its center to determine if measurable aerosol fallout was present. No flow was drawn from these filters. These samples turned out to be negligible. Two of the four sampling boxes were located towards the rear of the TFA and two towards the front. Also, three filter samplers were placed in the rear of the challenge chamber and three in the front. All sampler types and locations are shown schematically in Figure 1 and their coordinate locations are given in Table 2. Filter samples were collected using care to avoid contamination. The filters were removed and placed separately in 50 mL, screw cap, plastic centrifuge tubes to which 20 mL of recovery solution was added. Recovery solution consists of deionized water with a small amount of 14.3 N ammonium hydroxide added in a ratio of 1000 mL of water to 1 mL of ammonium hydroxide. Ammonium hydroxide was used to adjust the pH to between 9 and 10 for maximum fluorescence of the sodium fluorescein. The filter with recovery solution was mildly agitated to extract the sodium fluorescein, which is water soluble. Mild agitation of the filter in solution is recommended to minimize filter disintegration as the glass fiber fragments adversely affect fluorescence measurement. Fluorescence measurements were achieved with a laboratory fluorometer using a 492 nm excitation filter appropriate for sodium fluorescein. Calibration curves were prepared and used for extrapolation of concentration as grams of fluorescein per milliliter. The measurements were adjusted for solution volume, sample flow rate, and time to yield grams of fluorescein per liter of air.

4. RESULTS

Data are presented for monitoring aerosol concentration trends using three real-time generic particle counters and four mass monitors. The three aerosol particle counters included the APS 3321, AeroTrak 9350, and ELPI, while the four aerosol mass monitors included the APS 3321, DustTrak 8530, TEOM Model 1200, and ATI 2H photometer. The APS 3321 functioned as a particle counter and a mass monitor.

The real-time generic detection devices (the APS 3321, DustTrak 8530, TEOM Model 1200, and ATI 2H photometer) proved useful for monitoring concentration trends as a function of time, yet provided little benefit for quantitative purposes because of calibration issues and lack of discrimination as a consequence of generic detection. Collection on 47 mm filters and fluorescence measurement provided the desired discrimination between the fluorescent challenge and ambient background.

4.1 PAO Data

Table 3 summarizes the results of the PAO testing. Using the M28 SCPE blower pack with its single HEPA quality filter, a three test average PF was calculated at 77,458 with a sample standard deviation of 5,240. Such a low PF was not unexpected as HEPA filters are only 99.97% efficient at 0.3 μm, which is close to the PAO particle size of 0.528 μm.

Table 3. Summary Comparison of PAO Trials in October 2009. The aerosol mass concentrations (milligram per cubic meter) were measured in real-time using the ATI 2H

	1-Oct-09	2-Oct-09	5-Oct-09	5-Oct-09	6-Oct-09	6-Oct-09
	********	*Single HEPA* **********		*Single HEPA with controlled Leak*	*Double HEPA*	*Double HEPA with Controlled Leakage*
TFA Background	1.42E-04	6.48E-06	1.33E-05	6.67E-06	0.00E+00	0.00E+00
Challenge Background	2.44E-04	2.57E-03	5.62E-05	-	2.14E-03	-
time period	16:13-16:20	11:12-11:24	11:16-11:31	12:00-12:20	10:30-10:45	10:30-10:45
TFA Challenge	5.84E-04	5.08E-04	5.05E-04	1.01E-02	***1.0E-04	7.24E-03
Chamber Challenge	3.69E+01	3.67E+01	3.73E+01	2.04E+00	2.47E+01	2.05E+01
time period	*16:19-16:34*	*11:24-11:39*	*11:31-11:46*	*12:20-12:30*	*10:45-11:00*	*11:00-11:10*
TFA (Background Corrected)	4.42E-04	5.02E-04	4.92E-04	1.01E-02	1.00E-04	7.24E-03
Challenge (Background Corrected)	3.69E+01	3.67E+01	3.73E+01	2.04E+00	2.47E+01	2.05E+01
** PF Value (BK-cord)	83,313	73,228	75,833	202	246,817	2,826
Average PF Value (BK-cord)		77,458				
Std Deviation PF Value (BK-cord)		5.23E+03				

* Mass concentrations were monitored with an Air Technologies International (ATI) model 2H aerosol photometer calibrated for 100mg/m^3 PAO full scale. Two photometers, one for continuous challenge concentration and one for continuous TFA concentration, were used.

** Protection factor (PF) = Average Chamber Challenge Concentration (background corrected)/ Average TFA Challenge Concentration (background corrected)

*** Note: no aerosol detected in TFA during challenge period. A Lower Limit of instrument detection of 0.0001mg/m^3 was applied and averaged over each measurement for the sampling period to avoid division by zero in the PF calculation. Consequently, the PF is a conservative estimate based on the lowest level of detection (LLD).

The number and mass size distributions portrayed in Figure 7, as reported by the APS 3321 with a TSI diluter of 100:1 at 1134 h on 5 October 2009, illustrate the limited utility of the APS for monitoring PAO. The manufacturer reported NMD and MMAD for PAO at 0.245 and 0.528 μm, respectively, are below the sizing capabilities of the APS 3321 at 0.523 μm. Still, a portion of the polydispersed PAO aerosol was recorded and can be used as a relative trend. Careful selection of aerosol instruments used in testing is paramount to ensure compatibility with the target analyte according to the manufacturer's specified dynamic size range and counting rate limitations. The AeroTrack model 9310 generic particle counter, with a lower size limit of 0.3 μm is more suitable to monitor the submicron PAO.

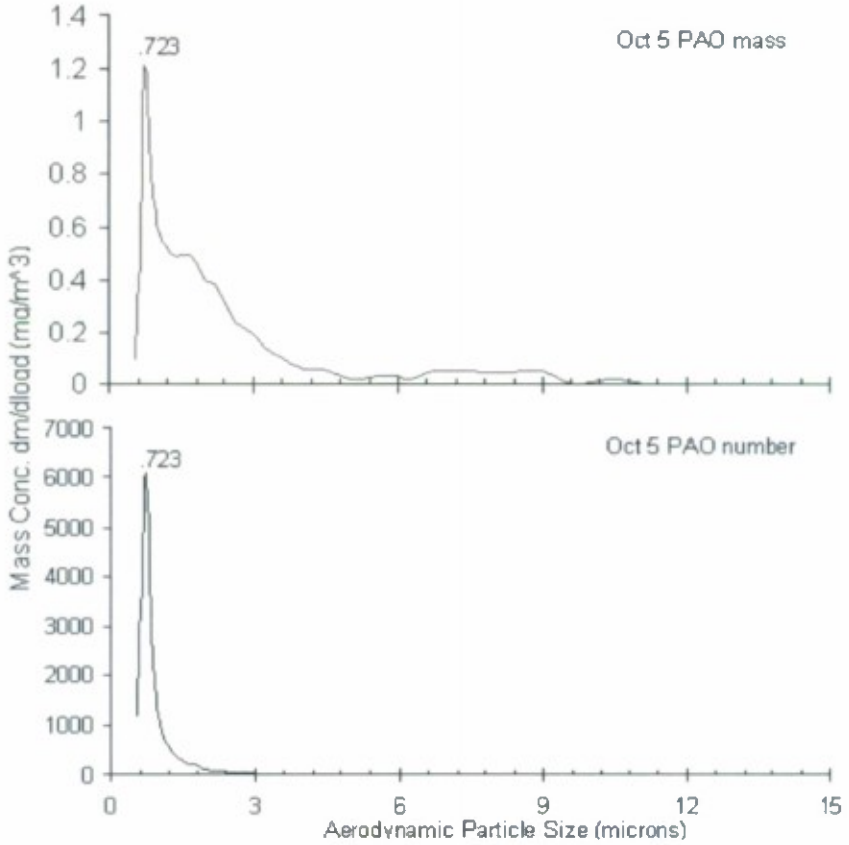

Figure 7. APS 3321 mass and diameter distributions, as a function of particle diameter, for PAO challenge aerosol at 1134 h on 5 October 2009.

Although the TSI AeroTrak reduced particle size detection limit allows it to be used to capture particles as small as 0.3 μm, the absence of an available dilutor for high concentration aerosols necessitates its use for inside the TFA only. Figures 8 and 9 show the number of particles (counts per liter) as a function of time. As shown in Figure 8, the count rate inside the TFA is in the range of the maximum count rate of 14,000 counts/L for AeroTrak. Above this limit, coincidence errors are encountered because too many particles occupy the detector's sampling volume at the same time. Either the particles are misplaced in erroneous size channels or the data is discarded. In Figure 8, only the lower particle size ranges or channels have been shown as no particles were detected in the upper channels of 1–3 and 3–5 μm. The high population of 0.3–0.5 and 0.5–1.0 μm particles penetrating the TFA of the M28 SCPE blower pack with the single HEPA quality filter should be noted.

To determine if particle penetration was through the single HEPA filter or from other sources, a second HEPA quality filter was placed in series, downstream of the M28 SCPE blower. Thus, pressurized clean air, taken from the challenge chamber and entering the TFA, had to pass through two HEPA filters. No evidence of aerosol penetration inside the TFA was observed when the results with the double HEPA filter system (Figure 8 bottom) were compared to a corresponding challenge period with the single HEPA filter (Figure 8 top). The challenge period activity with a double HEPA system from 1045 to 1100 h was below 1 count/L in all channels, which was the same for the background collection period from 1030 to 1045 h in Figure 8. This demonstrates that the main penetration path for the submicron PAO was through the single HEPA filter and not through the air lock or sample portals. This also indicates that a simple expedient in the field, if a submicron aerosol challenge is encountered, is a second HEPA filter added to the M28 SCPE, which would greatly reduce the threat.

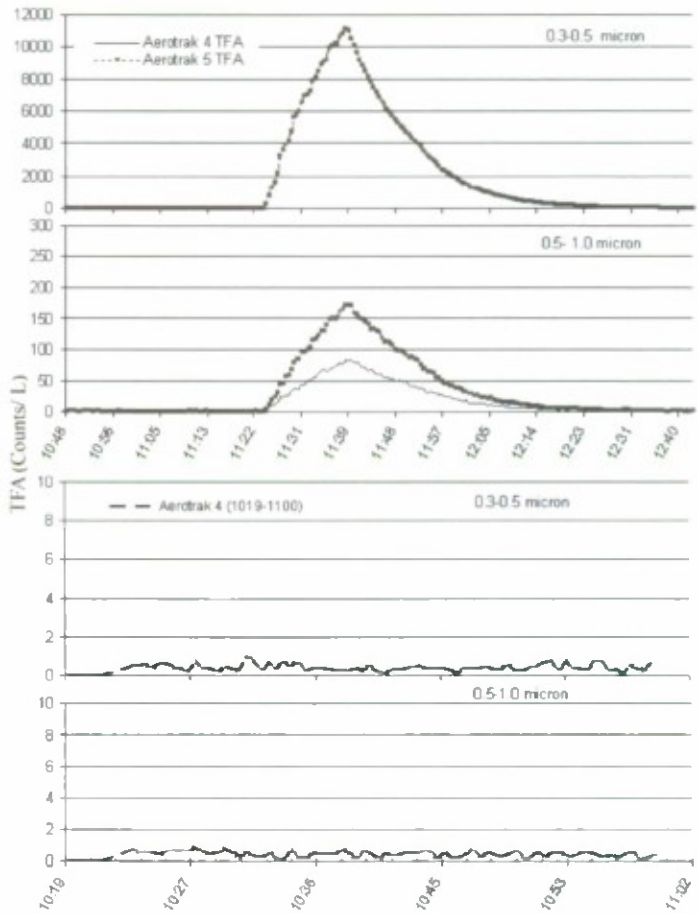

Figure 8. PAO submicron aerosol challenge penetration as monitored by AeroTrak 9310 for the two smallest size bins (0.3–0.5 μm and 0.5–1.0 μm) using a single (top, 2 October 2009) and dual (bottom, 6 October 2009) HEPA-protected shelter.

Figure 9 includes data from 1100 to 1110 h on 6 October 2009 showing the introduction of a "controlled leakage" directly into the TFA, bypassing the two HEPA filters, whereupon all particle channels show a sudden and dramatic increase in counting. A controlled

leakage is useful for verifying that instrumentation within the TFA is functioning. This would be essential for shelter systems having PFs exceeding 5,000,000.

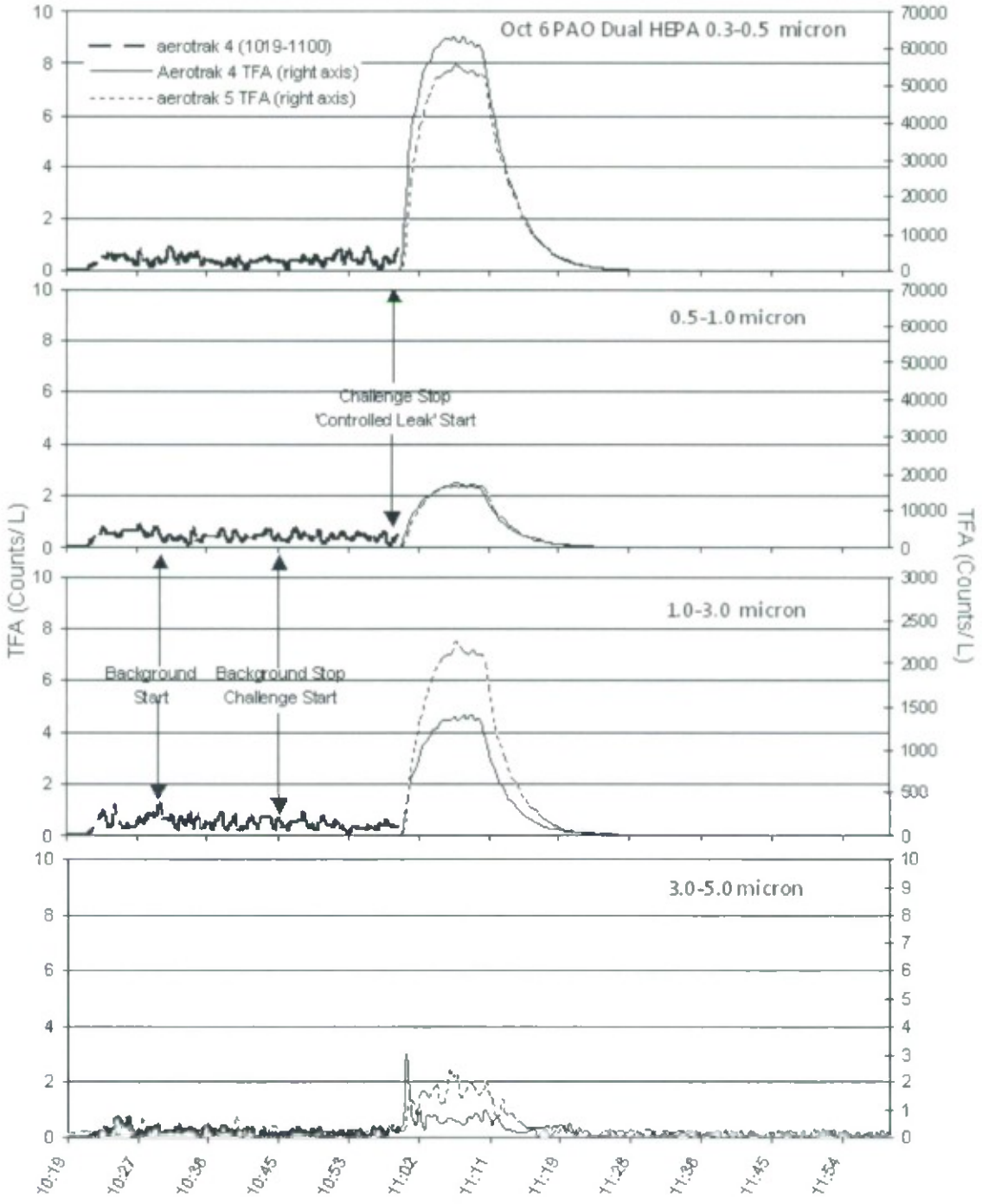

Figure 9. Aerotrak number concentration inside the TFA, as a function of time, for PAO aerosol using dual HEPA filters on 6 October 2009. Measurements were taken for aerosol background from 1030–1045 h; aerosol challenge from 1045–1100 h; and controlled leakage of aerosol from 1100–1110 h. Only controlled leakage gives evidence of challenge particles inside the TFA.

The ELPI, with a broad particle size range including seven submicron particle channels and a lower size limit of 0.04 μm, is particularly suited for monitoring the submicron PAO-nebulized aerosol. Figure 10 shows the particle size distribution (1140 h on 2 October 2009) that penetrated the PAO through the single HEPA filter of the M28 SCPE. As expected, concentration was significantly below 0.3 μm. The time series plot in Figure 11 portrays the marked disparity in penetration on a total number of particles per cubic centimeter between the submicron PAO at 1130 h and the larger 3 μm fluorescein at 1430 h performed on the same date. The count rate for the PAO submicron aerosol is significantly higher than that of the sodium fluorescein aerosol, even after factoring in the greater amount of PAO generated. This too is not unexpected as the PAO submicron aerosol is nebulized around the same size as the maximum penetration window through the HEPA filter at 0.3 μm, while the sodium fluorescein aerosol has a mass medium of 2.4 μm, so that it has only a small percentage by weight in the submicron range.

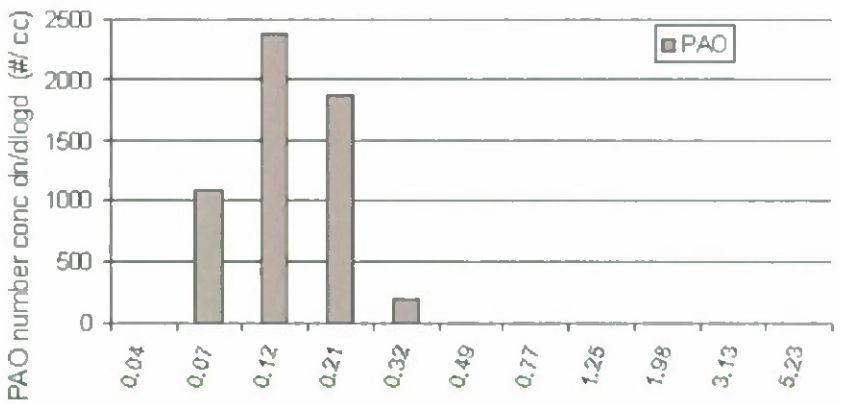

Figure 10. ELPI results for PAO submicron aerosol of 2 October 2009 at 1140 h showing penetration into the TFA through the M28 SCPE single HEPA filter.

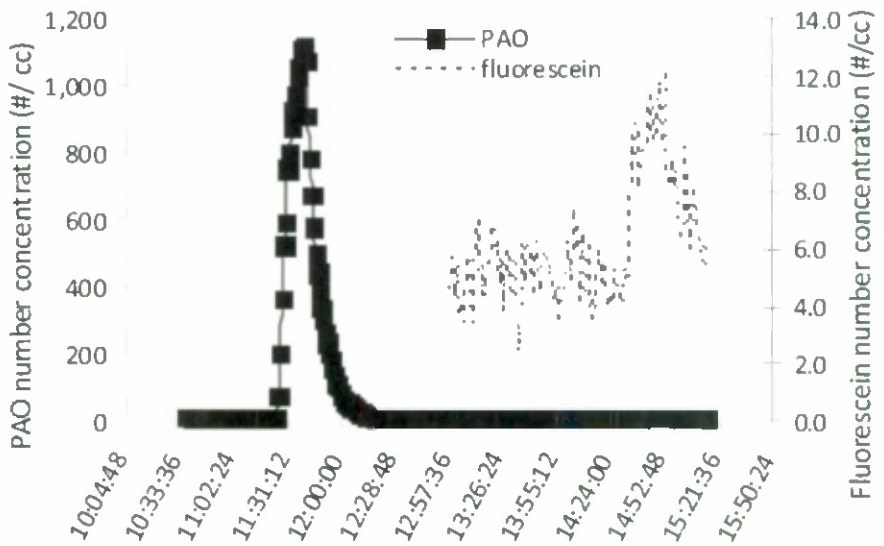

Figure 11. ELPI comparison of the total number of particles in the TFA using a single HEPA filter, as a function of time, for PAO and sprayed sodium fluorescein on 2 October 2009.

Figure 12 shows the result of introducing a controlled leakage, which bypassed the double HEPA filters, into the TFA with PAO aerosol. Data was taken on 6 Oct 2009 at 1111 h. Notice the slight shift to an increased size as the HEPA filters were bypassed. A controlled leakage is used to verify that the instrumentation inside the TFA functions. Unfortunately, there is no data for this date because the ELPI was not turned on at the time. However, from the Aerotrak data shown in Figure 9, it would seem reasonable to assume that the ELPI may have shown very little penetration.

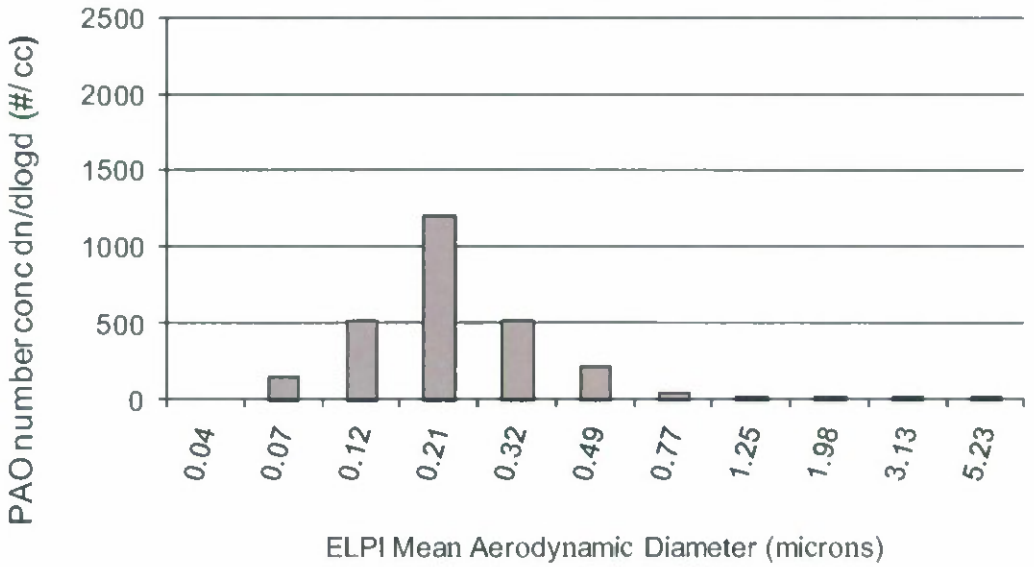

Figure 12. ELPI results for PAO submicron number distribution inside the TFA using dual HEPA filters on 6 October 2009 at 1111 h after introducing a controlled leakage of challenge aerosol.

Figure 13 shows a summary taken on 6 October 2009 overlaying total mass concentrations, as a function of time, for all devices used in monitoring PAO aerosol. The results shown include data collected with the ATI 2H photometer, APS 3321, DustTrak 8530, and TEOM Model 1200. The slight and maximum responses, observed earlier, for those samplers in the rear of the challenge chamber correspond to the direction of dissemination (front to rear). This is reflective of the finite time it takes to mix the aerosol through the challenge chamber. Even with four room-sized mixing fans placed in each corner of the challenge chamber and pointing diagonally upwards, the generated aerosol still took approximately 1 min to surround the TFA. This lag period is visible with the mass concentration devices within the challenge chamber, depending on the location of the device.

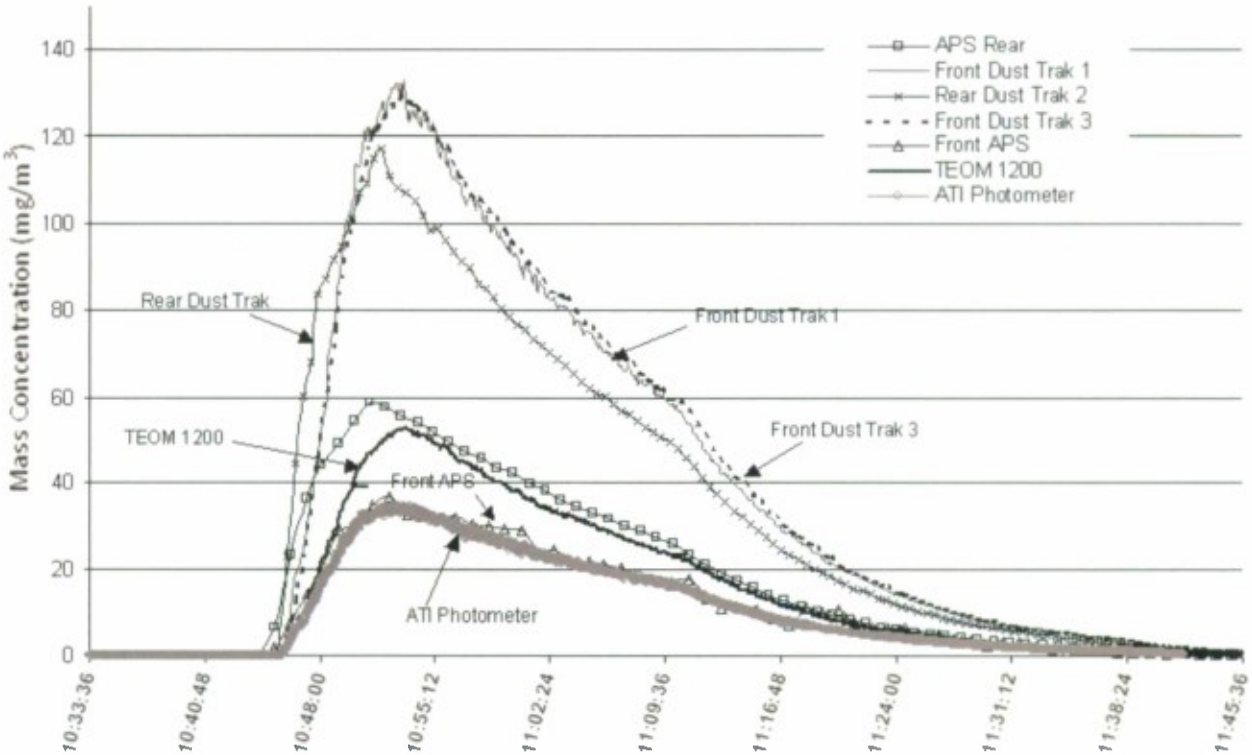

Figure 13. PAO submicron aerosol mass concentration as a function of time comparison between the DustTrak, APS 3321, and TEOM analyzers throughout the challenge chamber on 6 October 2009.

Only the ATI 2H photometer can be used as a standard for the mass concentration measurement as it was calibrated for the PAO submicron aerosol for the same aerosol generator used in these tests. Aerosol particle counters are generally acceptable for measurements of the particle size distribution, but they are not linear across wide variations in concentration and are shown here only for observing data trends. To be considered as a reference standard, the DustTrak would need to be calibrated for each aerosol material and particle size range for which it was used. The TEOM was also used for observing trends and was out of calibration. However, a calibrated TEOM would be an acceptable standard for the higher concentration aerosols in the challenge chamber.

4.2 Inert Aerosol Sodium Fluorescein Data

Upon dissemination, the resulting aerosol produced an MMAD of 2.4 μm with a log geometric standard deviation of 1.7 as measured with an Andersen 8-Stage Cascade Impactor. The data are summarized in Table 4 for each stage of the cascade impactor, illustrating the cumulative and percent cumulative mass concentrations. Figure 14 shows the cumulative percent mass as less than the particle diameter. The aerosol MMAD at 2.4 μm of the particle diameter is shown as a cumulative mass percentage of 50%. The logarithmic geometric standard deviation of 1.7 μm can be calculated as the 84.1% diameter divided by the 50% diameter.

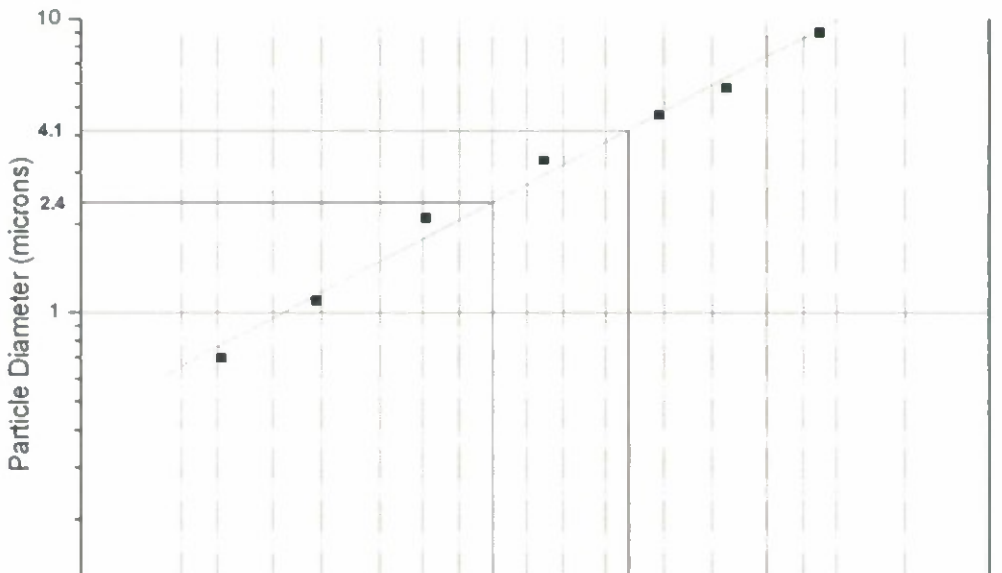

Figure 14. Log probability of the cumulative aerosol mass distribution of sodium fluorescein as collected by an Andersen 8-Stage Cascade Impactor, depicting an aerosol MMAD of 2.4 μm and a log geometric standard deviation (σ_g) of 1.7 μm.

The aerodynamic size distribution of the disseminated sodium fluoresecin, as measured by the APS depicted in Figure 15, displays a mode of 3.278 μm and a similar 2.4 MMAD as observed by the easeade impaetor.

Table 4. Caseade Impaetor Cumulative Pereent Aerosol Mass Coneentration of Sodium Fluoreseein as a Funetion of Aerodynamie Diameter. MMAD is 2.4 μm and the log geometrie standard deviation is 1.7.

Stage #	Size Range (μm)	Sodium Fluoreseein (g)	Mass (%)	Cum Mass (%)	Upper Bin Size (μm)
NV0	>9	7.34×10^{-5}	0.716	100.0000	
NV1	5.8–9.0	3.35×10^{-4}	3.267	99.2840	9.0
NV2	4.7–5.8	6.87×10^{-4}	6.700	96.0170	5.8
NV3	3.3–4.7	2.51×10^{-3}	24.498	89.3170	4.7
NV4	2.1–3.3	3.49×10^{-3}	34.040	64.8190	3.3
NV5	1.1–2.1	2.21×10^{-3}	21.569	30.7790	2.1
NV6	0.7–2.1	7.29×10^{-4}	7.104	9.2102	1.1
NV7	0.4–0.7	2.16×10^{-4}	2.106	2.1062	0.7
NV8	<0.4	---------- No Data ----------			0.4

Table 5 summarizes the results of the sodium fluoreseein testing, whieh eonsisted of one test using the M28 SCPE blower with its single HEPA filter, three tests with an additional HEPA filter in series with the M28 SCPE, and one test with the additional HEPA filter and a eontrolled leakage of the ehallenge aerosol into the TFA. The results of the M28 SCPE with its single HEPA filter are shown in Table 5 as yielding a PF of 2.52×10^5, whieh agrees with previous test results (Turetsky et al., 2009). The three tests using the additional HEPA filter yielded a PF average of 5.06×10^6 with a sample deviation of 3.48×10^5.

Thus, the single HEPA filter releases a suffieient number of the smallest partiele sizes from the sprayed sodium fluoreseein aerosol distribution into the TFA, whieh fails our eriterion of PF > 5,000,000, while the additional HEPA filter enables our goal to be met. Table 5 illustrates the intended purpose of the eontrolled leakage, allowing the verifieation of the funetioning equipment inside the TFA. The data in Table 5 was proeessed using eqs 2 and 3.

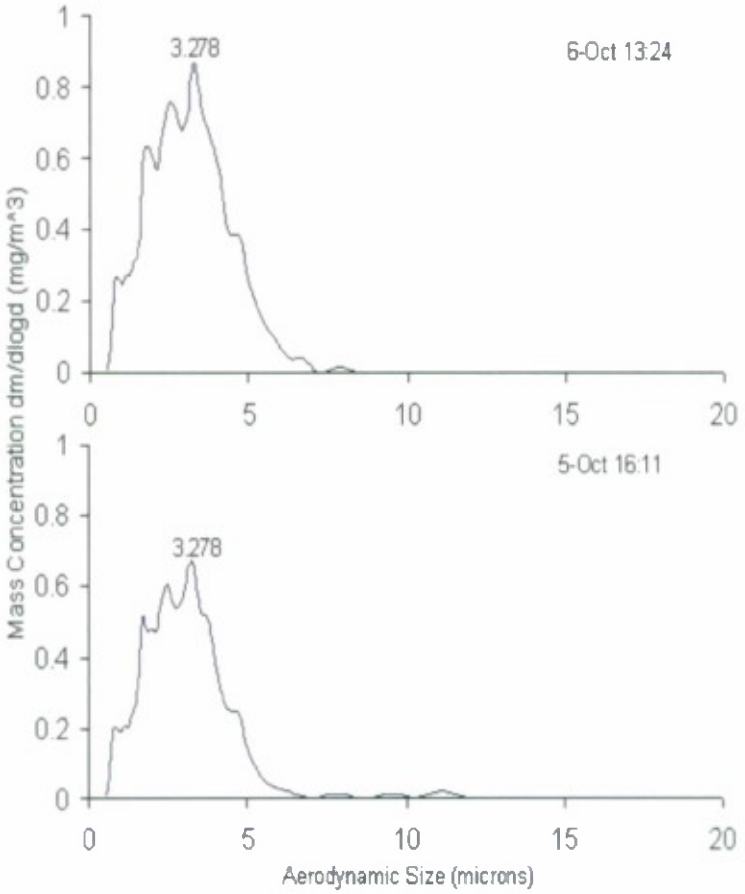

Figure 15. APS mass size distribution comparisons of sodium fluorescein in the challenge chamber as observed by two trials on 6 October 2009 at 1324 h (top) and 5 October 2009 at 1611 h (bottom). The results depict the polydispersed nature and submicron particle tails using Spraying Systems model SS1A nozzles.

According to eq 2, the PF is the quotient of the chamber challenge concentration, divided by the TFA challenge concentration, where the TFA challenge has been corrected for the TFA background.

$PF_{\text{dosage filters, background corrected}} =$

$$\sum_{j=1}^{p}\left(\frac{\left(\sum_{i=1}^{k}(m_i)_{\text{Chamber Challenge}}\right)/k}{\left(\sum_{i=1}^{l}\left((m_i)_{\text{TFA Challenge}}-(m_i)_{\text{TFA Background}}\right)/l\right)}\right)_j \Big/ p \qquad (2)$$

where m_i is the mass concentration measurement
k is the number of challenge chamber measurements
l is the number of the TFA measurements
p is the number of sample positions

From Table 5 and the example of 5 October 2009 with the single HEPA filter, we have

$$\left(\frac{(9.58+9.55+9.40)/3}{((4.93+4.83+4.89)\text{x}10^{-5})/3-((2.07+1.66+1.44)\text{x}10^{-6})/3}+\frac{(1.21+1.86+1.13)/3}{((4.89+4.81+4.74)\text{x}10^{-5})/3-((1.94+1.75+1.8)\text{x}10^{-6})/3}\right)\Big/2$$
$$= 2.5 \times 10^{5}$$

The considerations for the TFA background are as follows:

If (TFA challenge concentration – TFA background concentration) < 0 (i.e., negative)
or if (TFA challenge concentration – TFA background concentration) < LLD
where LLD is the lowest detectable level,

then we use eq 3

$$PF_{\text{dosage filters, LLD}} = \sum_{i=1}^{p} \left(\frac{\left(\sum_{i=1}^{k} \left(m_{i\ \text{Chamber Challenge}} \right) \right) / k}{\text{LLD}} \right)_i \Big/ p \qquad (3)$$

where i is the filter number in the chamber
k is the number of chamber filters
p is the number of locations

Let us again select an example from Table 5 and use the data for 6 October 2009, which is a double HEPA filter test. We have

$$\left(\frac{((1.41+1.38+1.4)\times 10^{1})/3}{3.07\times 10^{-6}} + \frac{((1.55+1.59+1.73)\times 10^{1})/3}{3.07\times 10^{-6}} \right) \Big/ 2$$

$$= 4.9 \times 10^{6} = PF_{\text{6 October 2009, LLD}}$$

Keep in mind that when using the LLD value, the calculated PF is a conservative estimate and therefore less than the actual PF.

Table 5. Summary Comparison of Sodium Fluorescein Filter Trials in October 2009

	Aerosol (Sodium Fluorescein) mass concentration (mg/m^3)				
	5-Oct	6-Oct	7 Oct AM	7 Oct PM	8-Oct
	Single HEPA	********** *Double HEPA* ********			*Double HEPA with Controlled Leakage*
Box 1 TFA Background Front - *Time=*	*14:19-14:49*	*11:53-12:23*	*11:50-12:20*	*16:33-17:03*	*10:55-11:25*
RF1	2.07E-06	1.29E-06	1.43E-06	1.55E-06	7.99E-06
RF2	1.66E-06	5.45E-07	1.15E-06	1.34E-06	1.32E-05
RF3	1.44E-06	1.09E-06	1.26E-06	5.19E-06	1.10E-05
average =	*1.72E-06*	*9.76E-07*	*1.28E-06*	*2.69E-06*	*1.07E-05*
*1 * Std Dev =*	*3.18E-07*	*3.86E-07*	*1.44E-07*	*2.16E-06*	*2.63E-06*
Box 2 TFA Background Rear - *Time=*	*14:19-14:49*	*11:53-12:23*	*11:50-12:20*	*16:33-17:03*	*10:55-11:25*
RF4	1.94E-06	7.48E-07	1.47E-06	1.52E-06	8.29E-06
RF5	1.75E-06	6.75E-07	1.37E-06	1.95E-06	3.79E-07
RF6	1.80E-06	7.94E-07	1.16E-06	4.12E-06	3.12E-06
average =	*1.83E-06*	*7.39E-07*	*1.33E-06*	*2.53E-06*	*3.93E-06*
*1 * Std Dev =*	*9.86E-08*	*6.04E-08*	*1.55E-07*	*1.39E-06*	*4.02E-06*
Box 3 TFA Sample Front - *Time=*	*15:57-16:27*	*13:10-13:40*	*13:18-13:48*	*17:20-17:50*	*12:00-12:30*
RF7	4.93E-05	1.08E-06	1.79E-06	5.53E-06	1.96E-03
RF8	4.83E-05	7.52E-07	1.29E-06	1.03E-06	1.99E-03
RF9	4.89E-05	5.71E-07	1.08E-06	1.20E-06	1.99E-03
average =	*4.88E-05*	*7.99E-07*	*1.39E-06*	*2.59E-06*	*1.98E-03*
*1 * Std Dev =*	*4.76E-07*	*2.56E-07*	*3.65E-07*	*2.55E-06*	*1.80E-05*
Box 4 TFA Sample Rear - *Time=*	*15:57-16:27*	*13:10-13:40*	*13:18-13:48*	*17:20-17:50*	*12:00-12:30*
RF10	4.89E-05	7.65E-07	3.99E-06	8.32E-07	2.44E-03
RF11	4.81E-05	1.24E-06	1.52E-06	8.87E-07	2.47E-03
RF12	4.74E-05	3.40E-07	1.39E-06	8.22E-07	2.42E-03
average =	*4.81E-05*	*7.81E-07*	*2.30E-06*	*8.47E-07*	*2.44E-03*
*1 * Std Dev =*	*7.60E-07*	*4.49E-07*	*1.47E-06*	*3.51E-08*	*2.41E-05*
RF 13 TFA air inlet	5.15E-05	1.57E-06	1.72E-05	1.06E-06	9.36E-04
Front Chamber					
RF14	9.58E+00	1.41E+01	1.46E+01	1.45E+01	1.48E+01
RF15	9.55E+00	1.38E+01	1.73E+01	1.44E+01	1.44E+01
RF16	9.40E+00	1.40E+01	1.55E+01	1.45E+01	1.46E+01
average =	*9.51E+00*	*1.40E+01*	*1.58E+01*	*1.45E+01*	*1.46E+01*
*1 * Std Dev =*	*9.96E-02*	*1.33E-01*	*1.41E+00*	*3.99E-02*	*2.33E-01*
Rear Chamber					
RF17	1.21E+01	1.55E+01	1.67E+01	1.66E+01	1.64E+01
RF18	1.86E+01	1.59E+01	1.80E+01	1.68E+01	1.63E+01
RF19	1.13E+01	1.73E+01	1.41E+01	1.63E+01	1.63E+01
average =	*1.40E+01*	*1.62E+01*	*1.63E+01*	*1.66E+01*	*1.64E+01*
*1 * Std Dev =*	*4.00E+00*	*9.32E-01*	*1.95E+00*	*2.62E-01*	*4.28E-02*
PF Front Samples (background	2.02E+05	-	-	-	7.40E+03
PF Rear Samples (background	3.02E+05	-	-	-	6.71E+03
PF average (background Correction) =	***2.52E+05***	-	-	-	***7.05E+03***
PF Front Samples (LLD)	-	4.55E+06	5.14E+06	4.71E+06	-
PF Rear Samples (LLD)	-	5.28E+06	5.29E+06	5.39E+06	-
PF Front & Rear average (LLD) =	-	4.91E+06	5.21E+06	5.05E+06	-
PF LLD Overall Average =		***5.06E+06***	Conservative Estimate		
PF LLD Overall std dev =		***3.48E+05***			

LLD = Lower Limit of Detection of 3.07E-06 mg/m^3 using 325 au at gain 200

PF = Protection Factor

Before we examine the particle size distributions entering the TFA, via either the single or double filter, let us examine Table 6 for aerosol uniformity in the challenge chamber during the sodium fluorescein aerosol dissemination. Table 6 shows a consistently higher aerosol mass concentration in the rear of the challenge chamber as opposed to the front. The variation runs from 3 to 25% with an average variation of 17%. Better mixing within the challenge chamber is needed. Also, the "clean air" exiting the entry/exit point is too close to the front filter dosage samplers. In hindsight, this systematic error can be dealt with easily in future tests and does not negate the successful development of the test protocol for testing COLPRO shelters with potential PFs up to 5,000,000.

Table 6. Challenge Chamber Uniformity during Sodium Fluorescein Disseminations in October 2009

Date (Oct 2009)	Comments (filters)	Background Time	Sample Time	Filter Loading (mg/m^3)		Ratio
				Front Chamber	Rear Chamber	
2	Single HEPA	1319–1349	1423–1459	6.69	8.85	0.76
5	Single HEPA	1419–1449	1557–1627	9.59	14.12	0.68
6	Double HEPA	1153–1223	1310–1340	14.05	16.44	0.85
7	Double HEPA	1150–1220	1320–1350	15.93	16.43	0.97
7	Double HEPA	1633–1703	1720–1750	14.56	16.79	0.87
8	Leakage	1055–1125	1200–1230	14.68	16.57	0.89

Typical APS 3321 results for the sodium fluorescein sprayed aerosol are shown in Figure 16, where aerosol mass concentration (milligrams per cubic meter) and number concentration are illustrated as functions of the aerosol particle diameter. Figure 16 shows the nearly identical aerosol distributions, taken in two distinct tests, on 5 and 6 October 2009, which demonstrate test repeatability. Note, the displayed sodium fluorescein mass distributions are very different from those of the PAO submicron aerosol shown in Figure 7. However, the similarities in number distributions, reported by the APS 3321 for the sodium fluorescein and PAO submicron aerosol, are attributed to the lower size limit of 0.52 μm of the instrument, which exceeds the majority of the PAO aerosol particles. Great care must be exercised in selecting aerosol-sizing equipment based on the expected size range and concentration to be encountered. A 100:1 aerosol diluter Model 3302A by TSI was used with the APS to bring the aerosol count rate below the coincidence error level set by the manufacturer at 1000 particles/mL. As shown in Figure 16, this coincidence level was exceeded somewhat, yet the data shows little evidence of coincidence counting, which often shows up as random particles at the large sizes, especially when plotted on a mass basis. The APS 3321 was not part of the equipment suite inside the TFA, because the aerosol particles that would be entering the TFA were correctly anticipated to be below its smallest detectable size.

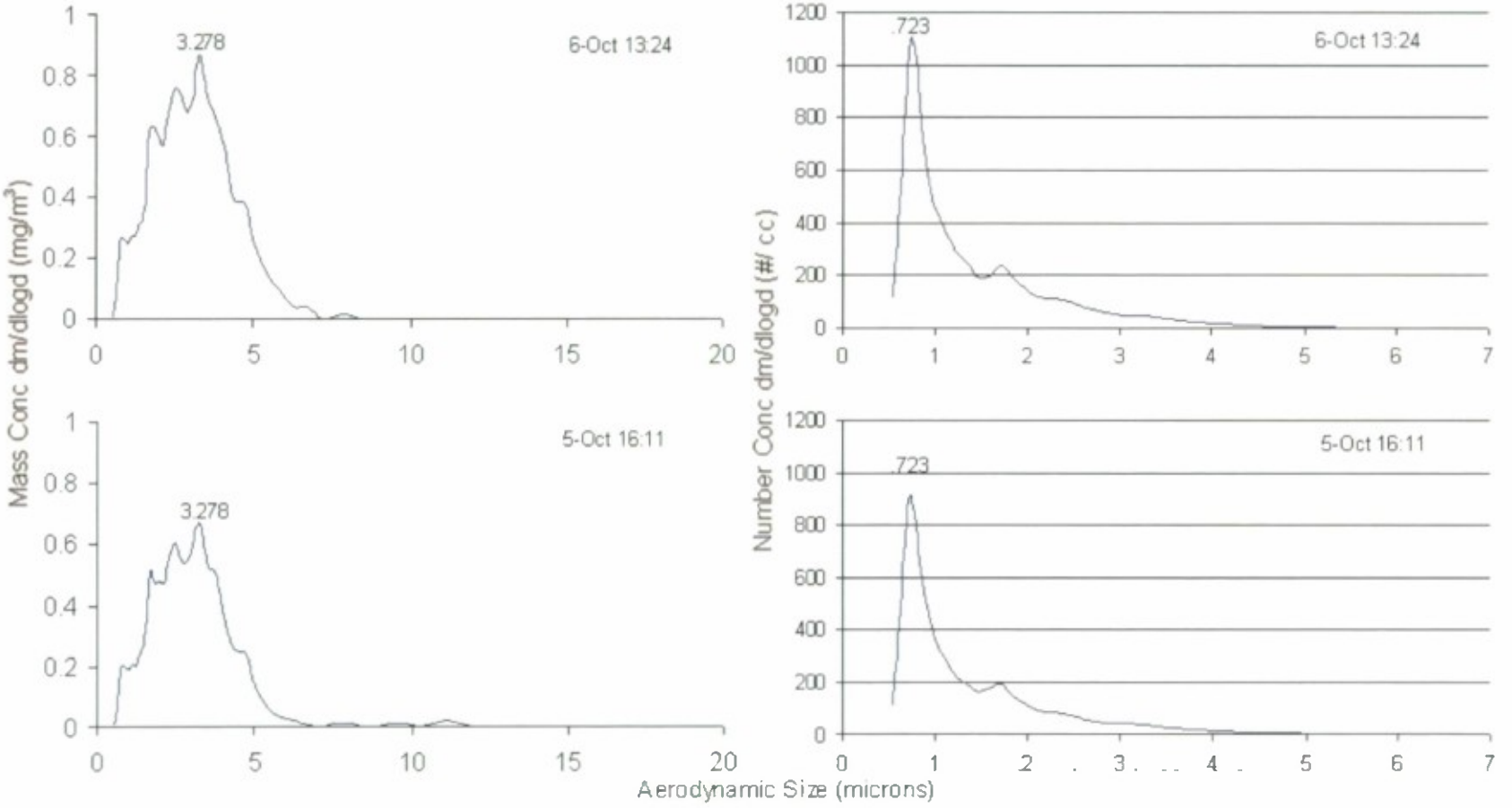

Figure 16. APS 3321 results for sodium fluorescein sprayed aerosol inside challenge chamber showing mass distribution (left) and number distribution (right) with data for 6 October 2009 at 1324 h (top) and 5 Oct 2009 at 1611 h (bottom).

Even with the 100:1 dilutor, the APS should not be used for absolute quantitative purposes, as it was designed and calibrated for particle sizing only. The counting efficiency of the APS 3321 is a function of particle size and extends from 40 to 60% for particle sizes from 0.4 to 8 µm, respectively, as discussed by Peters et al. (2003).

The generic particle detecting and counting attributes of real-time monitors is a concern when measuring COLPRO PFs. With their lack of ability to speciate, such counters cannot distinguish between challenge aerosol leaking into the TFA and the ambient aerosols already present. Methodologies incorporating fluorescent or tagged particles facilitating identification are an attractive alternative.

The TSI AeroTrak Model 9350 was used as a particle sizer/counter inside the challenge chamber and TFA. However, there is no diluter available for the AeroTrak. The AeroTrak coincidence counter level of 14,000 particles/L was easily exceeded during these high concentration aerosol tests; therefore, the data for the challenge chamber has not been presented in this report. Figure 17 shows the number of particles per liter for the smallest particle channels of the sodium fluorescein test of 5 October 2009, which uses the M28 SCPE with a single HEPA filter. All particle counts taken inside the TFA are well below the coincidence level. Evidently, the single HEPA filter is not adequate to remove all of the submicron particles in the sprayed sodium fluorescein. However, a quick comparison with Figure 8 (top) establishes that the sodium fluorescein penetration into the TFA is only a fraction (≈5% by number) of the penetration seen with the PAO submicron challenge of 2 October 2009. Figure 17 shows no particles above 1 µm inside the TFA during the challenge, similar to the PAO submicron test. This is another indication that the primary penetration/leakage into the TFA is through the filter and not the entry/exit point or any seams for the current static challenge tests.

By contrast, Figure 18 presents the AeroTrak particle number per liter count for the sodium fluorescein test of 7 October 2009, which used two HEPA filters. Note that there are no measureable particle counts inside the TFA in any of the particle channels during the sodium fluorescein aerosol challenge when the additional HEPA filter is added downstream of the M28 SCPE pack. This matches the results of the PAO submicron test challenge when using the double HEPA filters as seen in Figure 9.

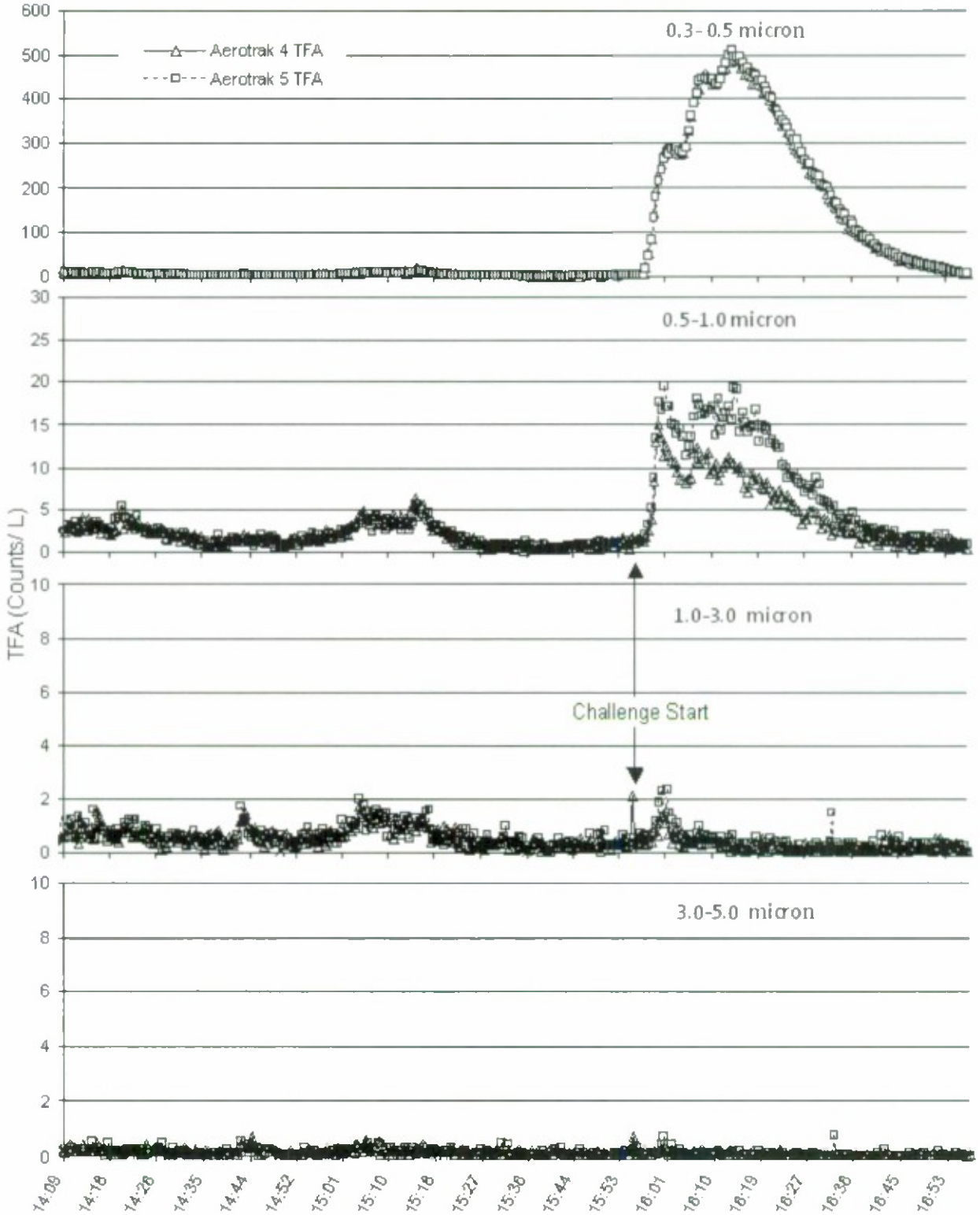

Figure 17. AeroTrak number concentration (counts/L) in the TFA as a function of time for the four smallest size channels of disseminated sodium fluorescein on 5 October 2009, which used a single HEPA filter. No particles above 3 μm are observed in the TFA for the aerosol challenge that commenced at 1557 h.

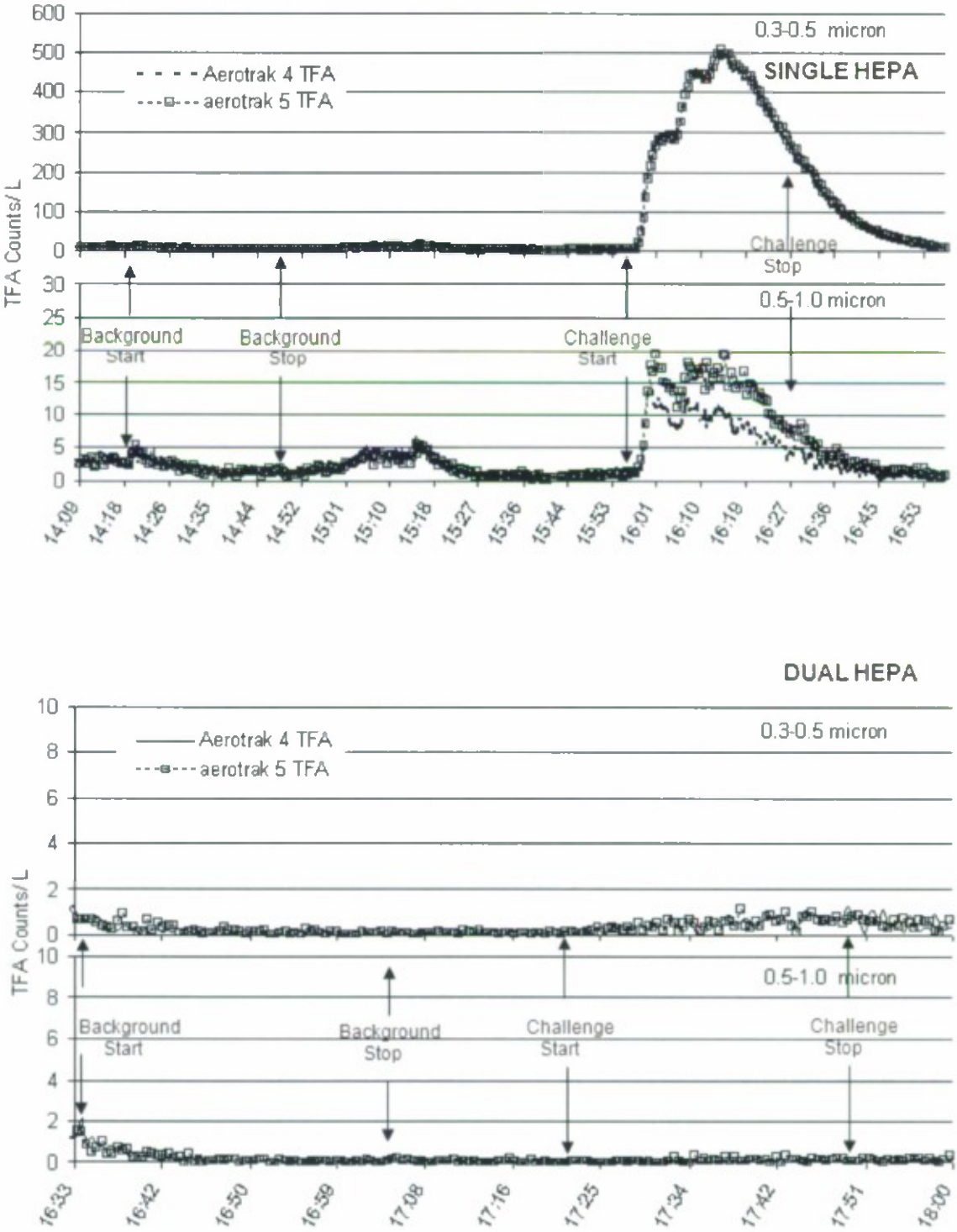

Figure 18. Sodium fluorescein penetration comparison as monitored by the AeroTrak 9350 for the two smallest size bins (0–0.5 µm and 0.5–1.0 µm) using single (top, 5 October 2009) and dual (bottom, 7 October 2009) HEPA-protected shelters.

Figure 19 presents the AeroTrak data taken inside the TFA using the double HEPA filter with a direct controlled leakage into the TFA to verify that the equipment functioned properly. With the direct injection of the challenge aerosol, 6.2 Lpm, all the particle channels from 0.3 to 5.0 μm detected aerosol.

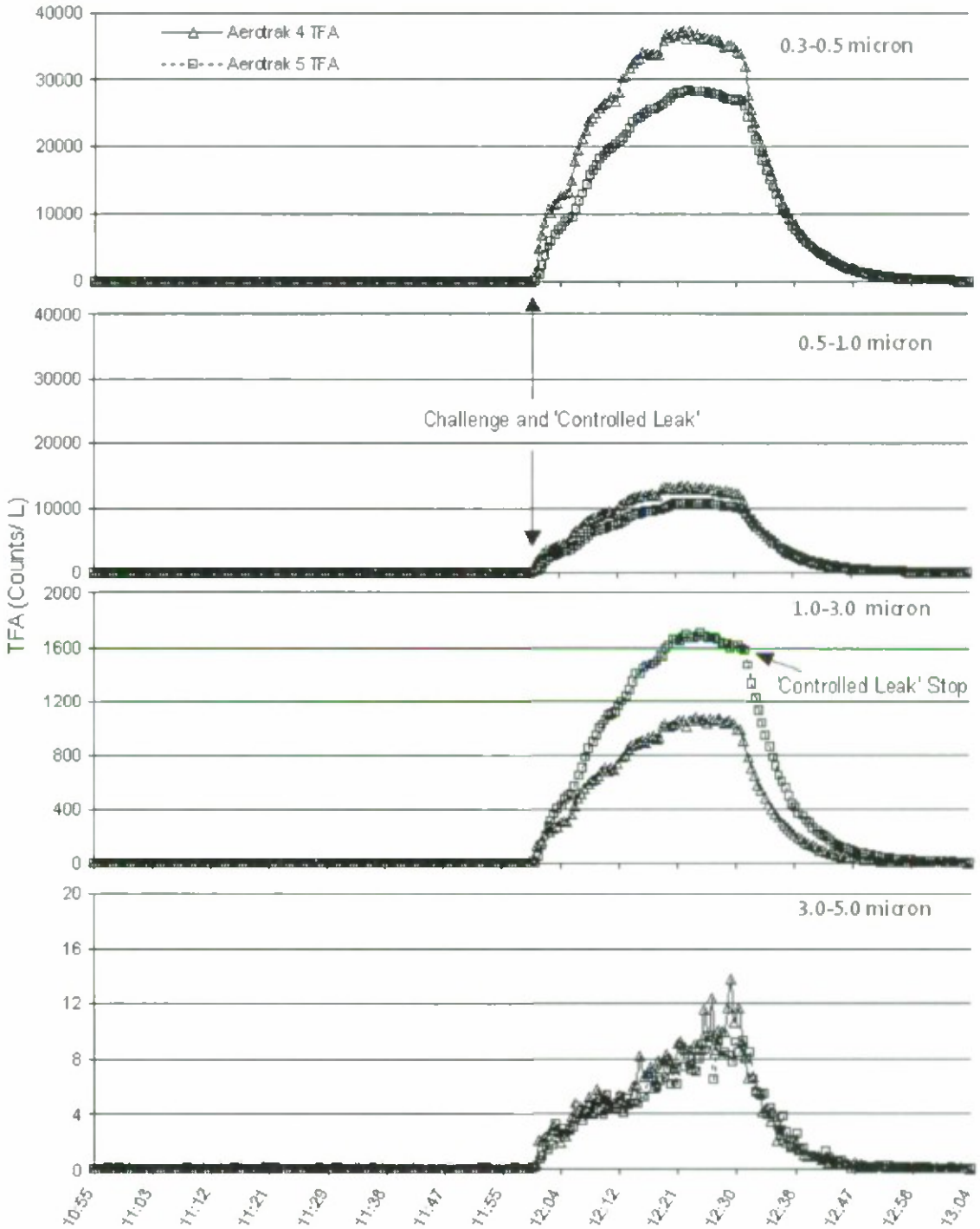

Figure 19. AeroTrak number concentration (counts/L) in the TFA, as a function of time, for the smallest particle size channels for the sodium fluorescein tests on 8 October 2009 with a "controlled" leak to bypass the dual HEPA filters. Challenge aerosol period of 1200–1230 h was also the "controlled" leak period (i.e., positive control).

The ELPI data for the sodium fluorescein trials is shown in Figures 20–23. Using a single HEPA filter, the number concentrations as displayed in Figure 20 are over twice those using the dual HEPA shelter as shown in Figure 21. Although it is perhaps not significantly different, the magnitude is less than the PAO submicron challenge in Figure 8. The emphasis is on the sprayed sodium fluorescein aerosol being a much coarser particle than the PAO aerosol, with most of it above the penetration window of the HEPA filter. The background aerosol, seen by the ELPI and shown in Figure 22, is not significantly different when using either the single or dual HEPA filters, which raises the question as to whether or not the particles shown in Figures 20 and 21 are from the challenge aerosol or from ambient background aerosol. More testing would be needed to clarify this point. A controlled leakage test is shown in Figure 23. The reported controlled leakage aerosol mass size distribution was much smaller than the disseminated challenge fluorescein aerosol of Figures 14 and 16, which can be attributed to the effects of the air eductor system used to aspirate challenge aerosol and inject it into the shelter. Nevertheless, the controlled leakage test verified that the aerosol monitoring equipment inside the TFA functioned properly.

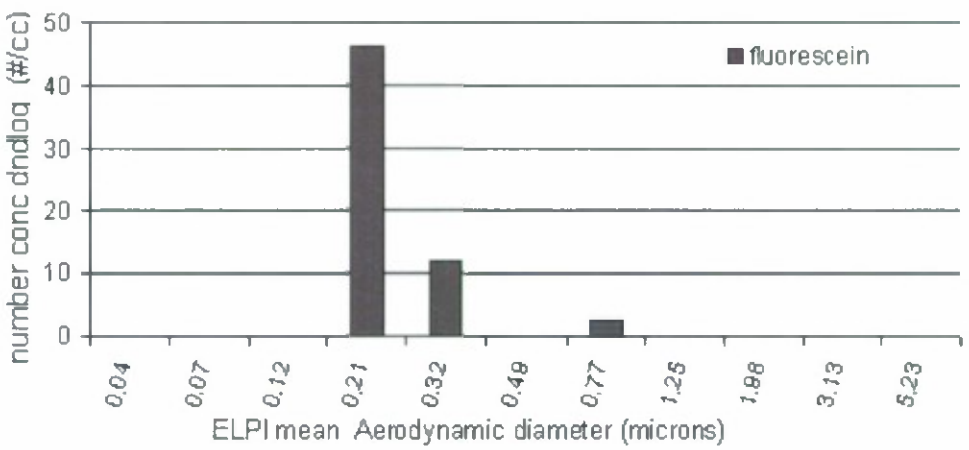

Figure 20. ELPI number concentration of sodium fluorescein, as a function of particle size inside the TFA, when using a single HEPA filter on 2 October 2009 at 1446 h.

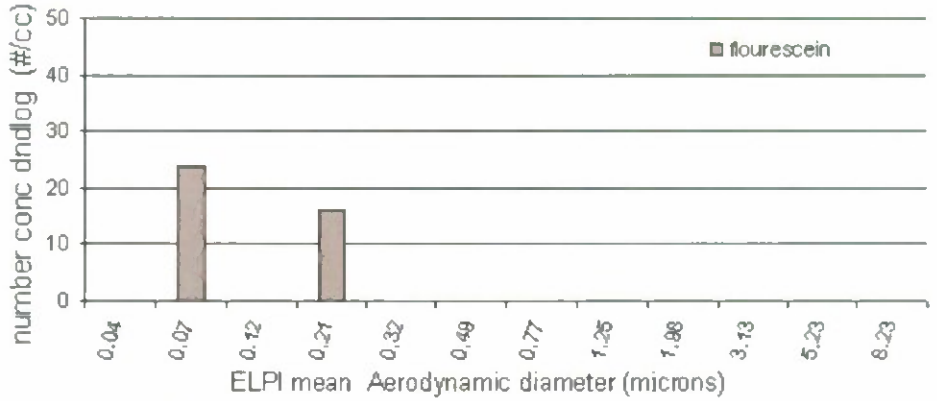

Figure 21. ELPI number concentration, as a function of particle size inside the TFA, using dual HEPA filters on 7 October 2009 at 1735 h.

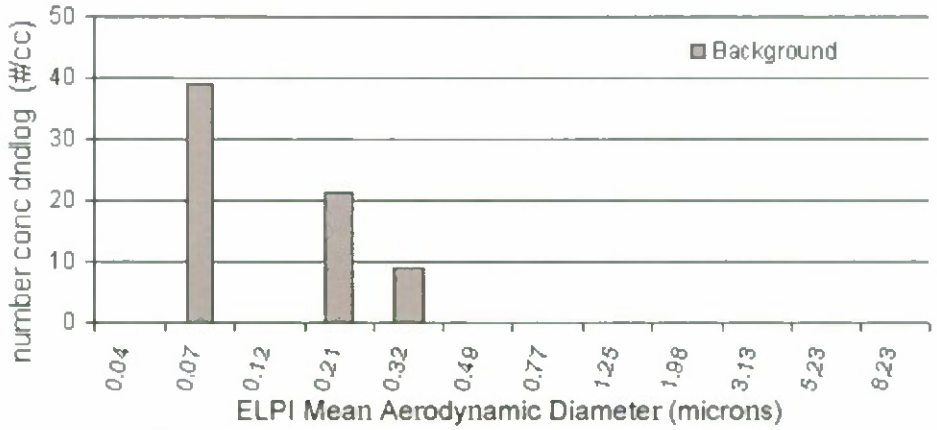

Figure 22. ELPI background number concentration, as a function of particle size inside the TFA, using dual HEPA filters on 7 October 2009 at 1228 h.

The sodium fluorescein aerosol mass concentrations were monitored using two APS 3321, three DustTrak, and one TEOM analyzers. The mass concentrations monitored as a function of time during the 7 October 2009 aerosol challenges are displayed in Figure 24. During the challenge, the aerosol generator operator attempted to maintain the aerosol mass concentration by pulsing the aerosol generation, as necessary, resulting in the observed oscillations. These perturbations are averaged during discrete 30 min collection periods by the dosage filters and they are used in the PF calculation. The slight earlier response times and maximal response observed for those samplers in the rear of the challenge chamber corresponds to the direction of dissemination (front to rear). This suggests better mixing techniques are needed. However, none of the APS, DustTrak, or TEOM data can be trusted because all these instruments are generic detectors and they were unable to differentiate between ambient and challenge aerosols. Furthermore, the DustTrak was not calibrated specifically for sodium fluorescein.

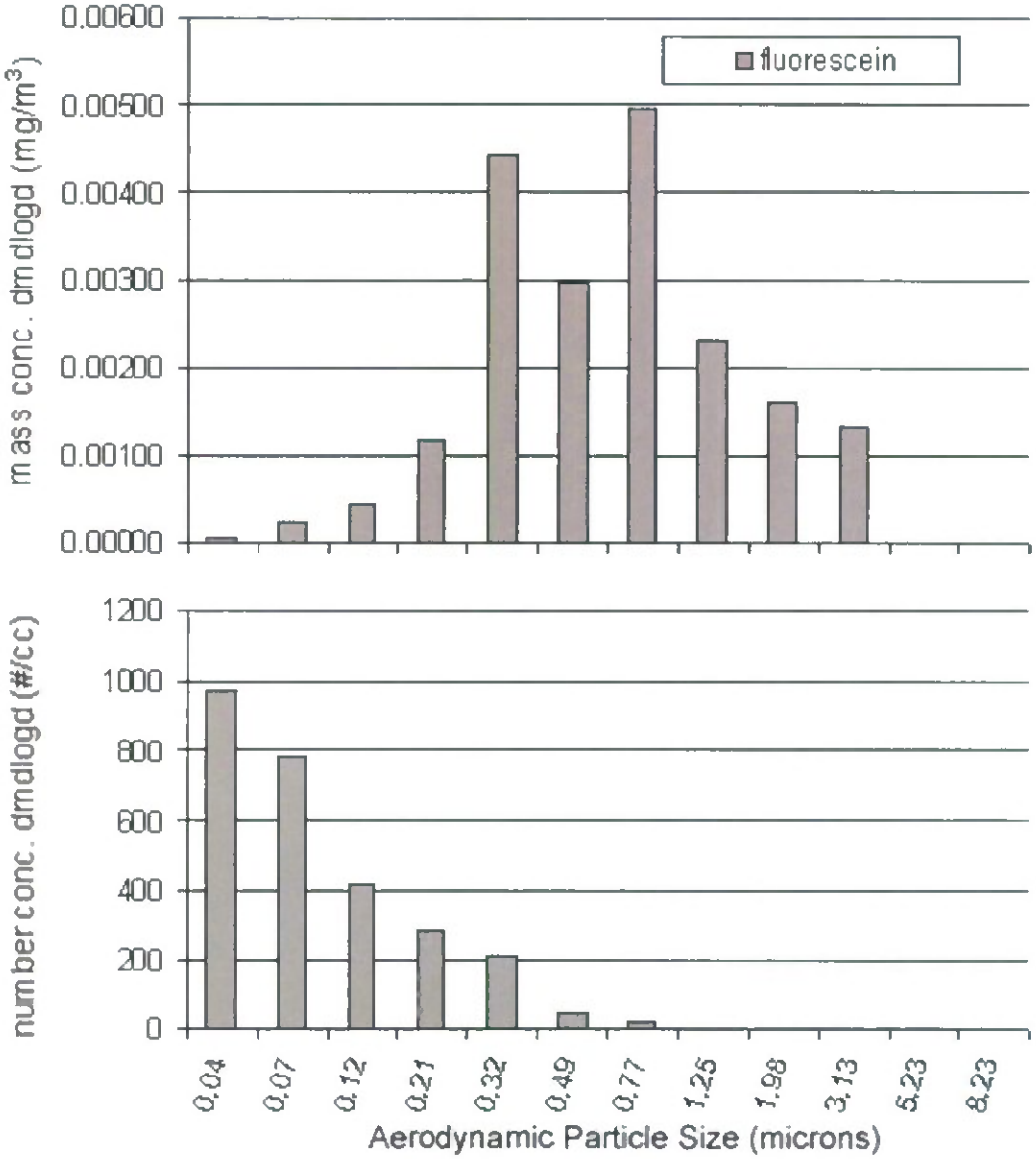

Figure 23. ELPI mass (top) and number (bottom) concentration, as a function of particle size inside the TFA, when using dual HEPA filters bypassed by a controlled leakage on 8 October 2009 at 1224 h, thus establishing that all equipment inside the TFA functioned.

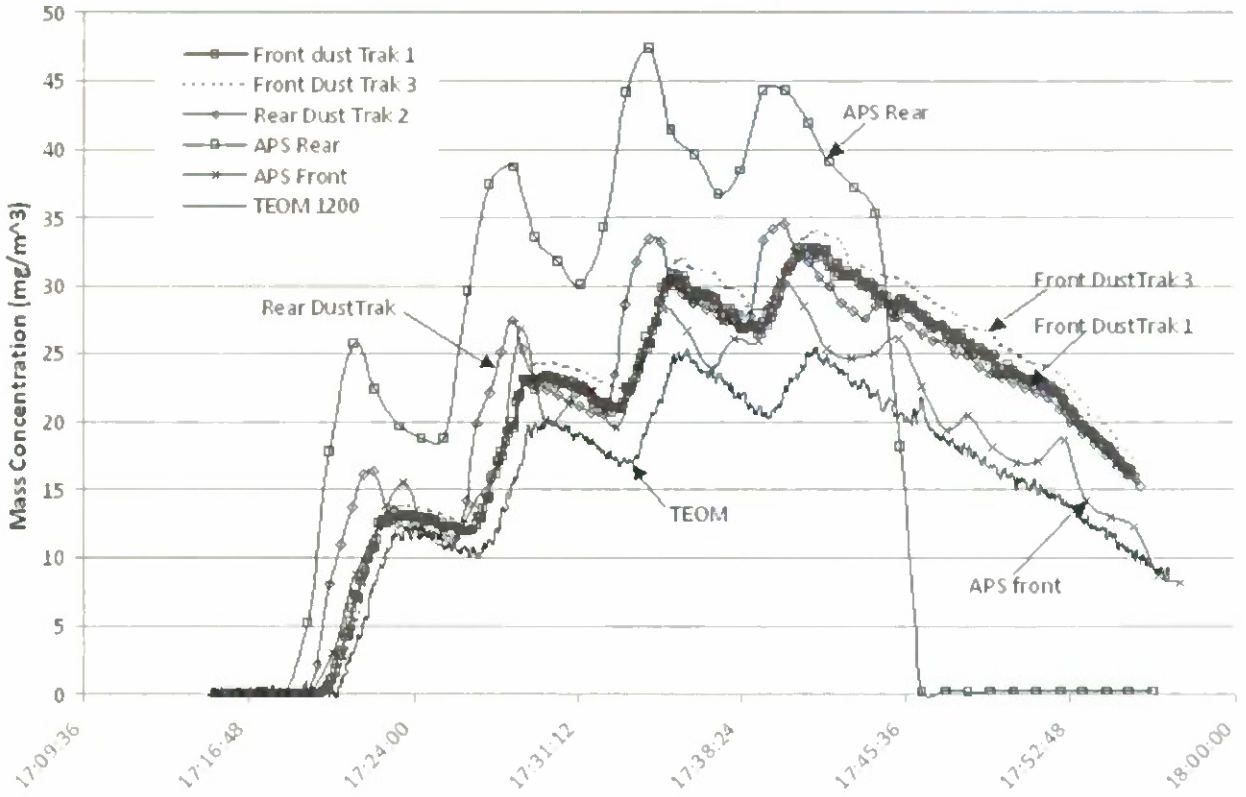

Figure 24. Challenge chamber monitoring of sodium fluorescein using multiple real-time aerosol monitors displaying total mass concentration (mg/m^3) as a function of time. Aerosol was monitored with three DustTrak, two APS 3321, and one TEOM analyzers on 7 October 2009.

5. DISCUSSION

A plausible basis for a COLPRO acceptance criteria and a PF determination for biological simulant and inert aerosol can be derived from the consideration of a challenge concentration and duration, the total volume of air breathed by an occupant inside the TFA, and the worst-case infectious dose. A typical resting Soldier breathes 32 L/min of air. A cloud of hazardous aerosol might reasonably persist for 60 min at a concentration of 2.5×10^7 biological organisms/m^3. The lowest infectious dose for the most probable biological agent to be used on the battlefield is 10 organisms for smallpox (*Variola major* causes smallpox). Therefore, the dose that personnel breathe in must not exceed 10 organisms/1920 L of air (32 L/min × 60 min). The PF is the quotient of the aerosol mass concentration outside the shelter divided by the aerosol mass concentration inside. Using 2.5×10^4 organisms/L of air as the challenge concentration and 9 organisms/1920 L (in the divisor), a minimum PF of 5.3×10^6 is obtained.

Because the shelters are maintained at a positive pressure, with all airflow being HEPA filtered, the aerosol concentration within the shelter is expected to be very low assuming that there is no internal activity (Eng et al., 1996). All of our measurements support this

hypothesis. However, with the measurement in the denominator of the PF calculation being numerically close to zero, the smallest error in measuring the inside aerosol concentration will result in a large variation to the resulting PF. A more stable measurement of the PF for the inert aerosols is possible with improved sampling sensitivity (i.e., by using an inert fluorescent aerosol and improved handling techniques to load and retrieve the samplers and place mechanical covers over the samplers when they are not in use).

Although the PF calculation in this test series using polydispersed aerosols focuses on concentration, the role of particle size should be considered and included in future tests because HEPA penetration is size dependant.

6. CONCLUSIONS

A submicron aerosol challenge of PAO was adapted from the filter testing community. This test provided a check on COLPRO shelter integrity, as well as being a quick, inexpensive method for uncovering major leakage. It also provided an estimate of submicron particle penetration. This method used a simple ratio of total aerosol mass concentration outside the TFA to that inside. The method did not provide a measure of the PF as a function of particle size.

The penetration of submicron particles was the major leakage path into our S280 shelter. This was demonstrated by the elimination of aerosol particle counts and fluorescence inside the TFA by the incorporation of an additional HEPA filter to the M28 simplified COLPRO equipment pack blower.

With the use of a sprayed sodium fluorescein aerosol (2.4 μm MMAD with logarithmic standard of deviation 1.7), a protocol for testing COLPRO shelters for a PF over 5,000,000 was developed. A simplified ratio of total aerosol mass concentration of all particle sizes outside the TFA to that inside was used, where the aerosol mass concentration was obtained using filter dosage samples and fluorometric analysis.

The PF was demonstrated to be a function of aerosol particle size as evidenced by the large change measured in the aerosol particle size distribution as it passed from the challenge chamber into the TFA.

None of the real-time aerosol particle size counters or aerosol mass monitors used in this series of tests should be used for real-time measurement of COLPRO shelter PFs with polydispersed aerosol challenges. Difficulties encountered in trying to do so included the following problems:

- The indicated count rate of current state-of-the-art particle counters can differ from the actual count rate. The indicated count rate has been reported to be a function of particle size.

- Particle counters need high order dilution to handle the very high concentration challenge aerosol outside the TFA, and they must operate without dilution inside the TFA. Of necessity, this requires a minimum of two particle counters; one for outside and one for inside the TFA. Particle counters exhibit differences in count rate even if they are from the same manufacturer. Calibration and correction factors must be determined to normalize the data. Aerosol dilution must be calibrated for particle size and demonstrated to be independent of aerosol concentration or established normalization factors.

- Current aerosol particle counters cannot distinguish between ambient background and challenge aerosol particles. This is very important at the lower levels of challenge aerosol penetrating the TFA. The situation is exasperated if there is any movement inside the TFA, which can generate its own set of background aerosol particles.

The use of real-time aerosol particle counters and aerosol mass monitors is appropriate for monitoring trends, if properly used within their dynamic range of operation.

7. RECOMMENDATIONS

Maintain the use of the industry standard complementary photometer and PAO pair for "quick check" of enclosure and filter leakage testing ONLY.

Further explore the use of fluorescent versus nonfluorescent monodisperse aerosols as small as 0.1 μm, as large as 2 μm, or having a narrow dispersion with a log geometric standard deviation of <1.2 for measuring PF. This should facilitate the discrimination against ambient background aerosols.

Further explore the use of real-time aerosol particle counters and aerosol diluters for measuring the PF of collective protection shelters as a function of particle size. Use high resolution aerosol size spectrometers and map their response as a function of particle size and count rate.

Explore better methods to improve mixing in large challenge chambers with high loadings and complex turbulency using a combination of different fan sizes, positions, and speeds.

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ACRONYMS

ABT	ambient breeze tunnel
APG	Aberdeen Proving Ground
APS	aerodynamic particle sizer
ATI	Air Techniques International
Bg	*Bacillus globigii*
COLPRO	collective protection
ECBC	U.S. Army Edgewood Chemical Biological Center
ELPI	electrical low pressure impactor
GPFU	gas/particulate filter unit
HEPA	high-efficiency particulate filter
IPE	individual protective equipment
LLD	lower limit of detection
L_g	log geometric standard deviation
L/min	liter per minute
MMD	mass mean diameter
MMAD	mass median aerodynamic diameter
MSDS	material safety data sheet
NMD	number median diameter
PAO	polyalphaolefin
PE	protective entrance
PF	protection factor
PSL	polystyrene latex spheres
SCPE	simplified collective protection equipment
SSIA	Spray Systems Corp.
σ	standard deviation
TFA	toxic-free area
TIC	toxic industrial chemical
TIM	toxic industrial material
ULPA	ultra-low particulate filter